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Role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain

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Abstract

The role of food-producing environments in the emergence and spread of antimicrobial resistance (AMR) in EU plant-based food production, terrestrial animals (poultry, cattle and pigs) and aquaculture was assessed. Among the various sources and transmission routes identified, fertilisers of faecal origin, irrigation and surface water for plant-based food and water for aquaculture were considered of major importance. For terrestrial animal production, potential sources consist of feed, humans, water, air/dust, soil, wildlife, rodents, arthropods and equipment. Among those, evidence was found for introduction with feed and humans, for the other sources, the importance could not be assessed. Several ARB of highest priority for public health, such as carbapenem or extended-spectrum cephalosporin and/or fluoroquinolone-resistant Enterobacterales (including *Salmonella enterica*), fluoroquinolone-resistant *Campylobacter* spp., methicillin-resistant *Staphylococcus aureus* and glycopeptide-resistant *Enterococcus faecium* and *E. faecalis* were identified. Among highest priority ARGs $bla_{\text{CTX-M}}$, bla_{VIM}, bla_{NDM}, $bla_{\text{OXA-48-like}}$, $bla_{\text{OXA-23}}$, *mcr*, *armA*, *vanA*, *cfr* and *optrA* were reported. These highest priority bacteria and genes were identified in different sources, at primary and post-harvest level, particularly faeces/manure, soil and water. For all sectors, reducing the occurrence of faecal microbial contamination of fertilisers, water, feed and the production environment and minimising persistence/recycling of ARB within animal production facilities is a priority. Proper implementation of good hygiene practices, biosecurity and food safety management systems is very important. Potential AMR-specific interventions are in the early stages of development. Many data gaps relating to sources and relevance of transmission routes, diversity of ARB and ARGs, effectiveness of mitigation measures were identified. Representative epidemiological and attribution studies on AMR and its effective control in food production environments at EU level, linked to One Health and environmental initiatives, are urgently required.

Keywords: antimicrobial resistance, food-producing environment, antimicrobial resistance genes, antimicrobial-resistant bacteria, animals, plants, aquaculture, environment, food

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Summary

The European Food Safety authority (EFSA) asked the Panel on Biological Hazards (BIOHAZ) to provide a scientific opinion on the role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain.

In particular, the Panel was asked:

1) to identify the main environmental sources and transmission routes leading to the contamination of foods of animal and non-animal origin with antimicrobial-resistant bacteria (ARB) and/or resistance determinants/genes (ARGs) (ToR1); 2) to identify the ARB and/or ARGs of highest priority for public health contaminating food through the environmental routes identified, as well as the main risk factors influencing their occurrence and persistence in food-producing environments and food (ToR2); 3) to review and, if possible, assess the impact of strategies and options to mitigate the risk of emergence, spread and food-borne transmission of those ARB (ToR3); 4) to identify data gaps influencing the assessment of food chain-related AMR risks posed by the environment and provide recommendations to inform future EU research priorities on this topic (ToR4).

For the purpose of this mandate, food-producing environments were defined as environments where food of animal or non-animal origin is produced or processed, at both preharvest (primary production) and post-harvest level (processing: e.g. slaughterhouses, processing plants). Three food sectors were considered: plant-based food production (fruits, vegetables and other crops), terrestrial animal production (poultry, cattle and pigs) and aquaculture (finfish, shellfish).

To address the mandate, a qualitative assessment was undertaken based on information from international reports, European Legislation, scientific literature (focusing primarily on European data for sources and occurrence of specific ARB/ARGs) and expert knowledge. Uncertainty was addressed in a qualitative manner following EFSA guidance. Food production sector maps, representing the sources of AMR and pathways for AMR dissemination in the different primary production and processing steps, were developed. ARB and ARGs of highest priority for public health in food-producing environments were defined based on international guidance documents and a consideration of the public health burden.

Food-producing sectors are linked with human sources and animal and environmental sources of AMR (ARB and ARGs) in a cyclical manner. These ARB and ARGs (as well as substances with antimicrobial activity) are introduced to animal- and plant-based food production environments, mostly through faecal waste (human and animal). Most of the sources identified also play a role as transmission routes. Fertilisers of faecal origin (e.g. manure), irrigation and surface water were identified as transmission routes of faecal ARB/ARGs of animal and human origin for plant-based food. Potential sources for this sector include soil, dust, farm animals, wildlife, arthropods, workers, equipment and process water. For terrestrial animals, feed, farm workers, air/dust, rodents, equipment and visitors, were identified as sources. Pastures, soil, surface water, drinking water, air, dust, wildlife or other domestic animal species are other potential sources of higher relevance for animals kept outdoor as compared to closed facilities. For aquaculture, water, sediments and feed were identified as sources. Wildlife, workers, ice and equipment were considered potential sources.

Based on expert knowledge, fertilisers of faecal origin (e.g. manure), irrigation and surface water are major sources and transmission routes of contamination for plant-based food. For terrestrial animals, the published evidence did not allow the importance of most of the sources identified to be determined, although, limited circumstantial evidence points to feed and, to a lesser extent, humans, as important sources/transmission routes. For aquaculture, water is the main transmission route.

Resistance to last resort antimicrobials was identified in bacterial pathogens (highest priority Group 1 bacteria) and in commensals or environmental bacteria encoded by ARGs carried on mobile genetic elements (highest priority Group 2 bacteria) in the food production sectors investigated. Among the first highest priority ARB group, carbapenem (CP-R)/extended-spectrum cephalosporin (ESC-R)/ fluoroquinolone resistant (FQ-R) and/or MDR *Salmonella enterica*, ESC-R/MDR Enterobacterales, FQ-R *Campylobacter* spp., methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) were detected. Among the second group, ESC-R and/or FQ-R *E. coli* and *Klebsiella pneumoniae* were commonly reported. Mobile ARGs conferring ESC-R and colistin-R in *E. coli,* CP-R in *Acinetobacter* spp. and MDR in Enterobacterales were also reported. Glycopeptide-R in *E. faecium* or *E. faecalis*, as well as oxazolidinones-R enterococci were also identified. These highest priority bacteria have been isolated from a range of sources, including manure, water, workers and wildlife at primary production, as well as transport, lairage, slaughter and meat processing at post-harvest level. Among

the highest priority ARGs, those conferring resistance to CPs (e.g. *bla*$_{\text{VIM}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{OXA-48-like}}$, *bla*$_{\text{OXA-23-like}}$), ESCs (e.g. *bla*$_{\text{CTX-M}}$, AmpC encoding genes), plazomicin (*armA*), colistin (*mcr* genes), methicillin (*mecA, mecC*), glycopeptides (*van*A genes) and oxazolidinones (*cfr, optrA*) have been reported.

Several factors can contribute to the occurrence and persistence of ARB/ARGs in food-producing environments: selective pressure in the animal and environmental microbiomes (use of antimicrobials, heavy metals or biocides), continuous cycling of bacteria between animals and their environments, inadequate definition or implementation of biosecurity measures and, for post-harvest situations, food safety management systems (FSMS) with ineffective food hygiene procedures. Moreover, bacterial characteristics, such as resilience/stress response capability, biofilm formation, ARG transferability, ARG co-localisation with other ARGs or heavy metal/biocide tolerance genes in the same genetic platform, as well as mechanisms to minimise the ARGs' fitness cost may also be relevant.

Apart from prudent antimicrobial use (AMU), the most important measures to mitigate AMR applicable for all the food-production sectors investigated, both at pre- and post-harvest, involve the correct implementation of effective general measures (good hygiene practices, biosecurity) to prevent/reduce occurrence and transmission of pathogens and other microorganisms. Biological methodologies that focus specifically on the reduction/elimination of ARB in the food production sectors, such as CRISPR-Cas, phages, or predatory bacteria are in the early phases of research and development in the AMR field. Activities at production stages which can widely disseminate large numbers of ARB and ARGs in the different production sectors are a priority for intervention. For all sectors, reducing the likelihood of introduction, dissemination and persistence of faecal contamination is a priority. For plant production, reducing the bacterial content of manure, sewage sludge and irrigation water is important. In livestock production, preventing transmission from other animals (e.g. rodents, arthropods and wild birds), dust, feed or surface run-off water, as well as proper implementation of cleaning/disinfection, and hygienic procedures for workers are relevant. For aquaculture, ensuring high microbial water quality by, e.g. reducing/eliminating ARB and/or ARGs in wastewater effluents and measures to prevent feed contamination are priorities. In post-harvest stages, the implementation of FSMSs is currently the main mitigation and preventive strategy to minimise the risk. Mitigations directed to prevent ARB and ARGs in different water sources (e.g. irrigation water, surface water and fresh and marine water of aquaculture) include some advanced wastewater treatment technologies, reducing raw sewage discharges, improving conventional wastewater treatment or implementing a multiple barrier approach to protect plant production and aquaculture.

A large number of data gaps exist in relation to the sources and transmission routes of ARB and ARGs in food-producing environments, diversity of ARB and ARGs/MGEs and effectiveness of many mitigation measures. Despite a large number of studies that have investigated the occurrence of ARB and ARGs in livestock and food, the role played by the environment is not sufficiently researched, and there are insufficient data to support a specific assessment of the quantitative impact of contamination of the EU production environment on foods or public health.

Priorities, among the numerous recommendations for further research, include the need for One Health-based integrated studies, harmonised environmental AMR monitoring/surveillance strategies, long-term longitudinal cohort studies on highest priority ARB/ARGs and studies involving environmental exposure of food animals. The most urgent within this topic would be to optimise suitable sensitive and standarised methodologies for detection of ARB/ARGs, and define sampling strategies for the different producing environments. Validating the efficacy of practical mitigation methods (e.g. current biosecurity and hygiene-based control programmes, environmentally friendly water treatment methods) would also be recommended. Within this topic, the most urgent would be assessing and developing validated methods for decontamination, aimed at important highest priority ARB and ARGs in the production environment, heat treatment conditions for animal feed and treatment of faecal waste and wastewater used for fertilisation/irrigation or processing crops. These studies should be linked to assessment of the effect of future policy developments (e.g. within the EU Green Deal) affecting food-producing environments, AMU and climate change impacts.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Antimicrobial resistance (AMR) is currently a major global threat as recognised by all international organisations, including the WHO, with estimates of hundreds of thousands of human deaths annually worldwide, including about 33,000 annual fatalities in the EU alone.[1]

Policy makers are taking action against AMR across the globe. In 2011, the European Commission (EC) published its first Action Plan (covering the 2011–2016 period) against the rising threats from AMR[2] and identified seven priority areas for action against AMR, including the intention to contain the risks of spreading AMR via the environment. The evaluation of the 2011 EC action plan[3] highlighted the need to improve scientific understanding of the role played by the environment in the emergence and transmission of resistance through animal, human and manufacturing waste in water and soil, and to explore what action may be required to reduce associated risks. In 2017, the EC launched its second AMR Action Plan.[4] This included specific actions to make the EU a 'best practice region', including 'better addressing the role of the environment' and 'closing knowledge gaps on AMR in the environment'. The EC action plans were building on a One Health approach, i.e. addressing the threat from a holistic and transdisciplinary approach, considering human, animal and the environmental sectors.

Major international organisations such as WHO, FAO and OIE have also recognised the need to further discuss and investigate this matter.[5] Codex Alimentarius is reviewing and updating its standards and guidelines to ensure a more integrated and multidisciplinary approach to AMR.[6]

Humans can acquire antimicrobial-resistant bacteria (ARB) from many different sources and routes, including human-to-human transmission, direct contact with food-producing animals and pets, food-borne transmission and via the environment. In the last years, increasing importance has been attributed to the role of the environment as a source of antimicrobial-resistant bacteria/genes for both humans and animals, and to the need to tackle AMR from a One Health perspective. However, there are still large uncertainties in the knowledge on the actual role played by the environment in the emergence, spread and persistence of antimicrobial-resistant bacteria.

Food-producing environments are defined for the purpose of this mandate as all environments where food of animal or non-animal origin is produced or processed, at both primary level (e.g. animal farms, fruits and vegetables cultivation fields, etc.) and post-harvest level (e.g. slaughterhouses, processing plants, etc.). Food-producing environments can be contaminated by antimicrobial-resistant bacteria (including resistance determinants and mobile genetic elements) deriving from different environmental sources, such as for example:

- effluents (e.g. slurry, manure, air) from terrestrial/aquatic food-producing animals;
- effluents and other residues from post-harvest food plants (e.g. slaughterhouses and food processing plants);
- effluents from urban and hospital waste-water treatment plants;
- crop production and horticulture (due to direct use of antimicrobials).

Once antimicrobial-resistant bacteria contaminate food-producing environments, they can further spread throughout the food chain through several routes and eventually constitute a possible threat for public health.

Therefore, it is important to review the scientific evidence available on the main environmental sources leading to the contamination of food with antimicrobial-resistant bacteria and the routes through which antimicrobial-resistant bacteria can be transmitted throughout the food chain. It is also important that, based on the scientific information reviewed, the antimicrobial-resistant bacteria of public health priority transmitted through such routes are identified. A review of the existing or new strategies and control options to mitigate the risks deriving from those antimicrobial-resistant bacteria along the food chain would provide EU risk managers updated information on the options to manage AMR-related risks at environmental level and more in general to contribute to the fight against AMR.

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[1] https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(18)30605-4/fulltext

[2] http://ec.europa.eu/health/amr/sites/amr/files/communication_amr_2011_748_en.pdf

[3] https://ec.europa.eu/health/sites/default/files/antimicrobial_resistance/docs/amr_final-report_2016_rand.pdf

[4] https://ec.europa.eu/health/amr/sites/amr/files/amr_action_plan_2017_en.pdf

[5] http://www.who.int/foodsafety/areas_work/antimicrobial-resistance/tripartite/en

[6] http://www.fao.org/fao-who-codexalimentarius/thematic-areas/antimicrobial-resistance/en

To carry out the above tasks, it is proposed that EFSA, in accordance with Article 29(1)(b) of Regulation (EC) No 178/2002, under the leadership of the Panel on Biological Hazards (BIOHAZ Panel), undertakes a self-task mandate to produce a scientific opinion on the role played by the environment in the emergence and spread of AMR through the food chain.

With the aim of tackling the issue from a One Health perspective, it is suggested that during the preparation of the scientific opinion, EFSA consults other European Union Agencies, in particular the European Centre for Disease Prevention and Control (ECDC), the European Medicines Agency (EMA) and the European Environment Agency (EEA).

The EFSA BIOHAZ Panel is requested to deliver this scientific opinion 12 months after the start of the activity, and by 31 December 2020 at the latest. The deadline was subsequently extended to 30 April 2021.

- Terms of reference:

The BIOHAZ Panel is requested to address the following terms of reference:

A) To identify the main environmental sources and transmission routes leading to the contamination of foods of animal and non-animal origin with antimicrobial-resistant bacteria and/or resistance determinants.
B) Among antimicrobial-resistant bacteria and/or resistance determinants contaminating food through the routes identified above, to identify the ones of highest priority for public health, if possible their relative importance, and the main risk factors influencing their occurrence and persistence in food-producing environments and food.
C) To review and, if possible, assess the impact of existing or new possible strategies and options to mitigate the risk of emergence, spread and food-borne transmission of the antimicrobial-resistant bacteria identified above.
D) To identify data gaps influencing the assessment of the food chain-related AMR risks posed by the environment and provide recommendations to inform future EU research priorities on this topic.

1.2. Interpretation of the Terms of Reference

- The ToRs were discussed and clarified.

As indicated in the background to the mandate, food-producing environments are defined for the purpose of this mandate as environments where food of animal or non-animal origin is produced or processed, at both preharvest (primary production) and post-harvest level (processing: e.g. slaughterhouses, processing plants). The retail stage is not considered in the assessment.

The opinion focuses on antimicrobial-resistant bacteria (ARB) and/or antimicrobial resistance determinants, defined as the genes (including resistance resulting from mutations in housekeeping genes) encoding resistance to antimicrobials (ARGs), in EU food production systems, or, where applicable, in similar production systems from other regions of the world.

Three food sectors are considered in the opinion: plant-based production (production of fruits, vegetables and other crops), aquaculture (finfish and shellfish) and terrestrial animal production (poultry, cattle and pigs).

It is recognised that the usage of antimicrobials, or certain biocides and metals (AMU) is an important factor for the occurrence and further selection and spread of antimicrobial resistance (AMR) in food-producing systems. However, the assessment of the contribution of AMU to AMR in food-producing systems and measures to restrict their use will not be included, as these issues have been thoroughly addressed in other documents (e.g. EMA and EFSA, 2017).

Accordingly, **ToR 1** '*To identify the main environmental sources and transmission routes leading to the contamination of foods of animal and non-animal origin with antimicrobial-resistant bacteria and/or resistance determinants'* has been reformulated as assessment questions (AQs):

- **AQ1a.** What are the environmental sources and transmission routes for antimicrobial-resistant bacteria and resistance genes for the different food production sectors identified?
- **AQ1b.** What is the importance of the different sources and transmission routes of antimicrobial-resistant bacteria and antimicrobial resistance genes?

For **ToR 2**, '*Among antimicrobial-resistant bacteria and/or resistance determinants contaminating food through the routes identified above, to identify the ones of highest priority for public health, if* *possible their relative importance, and the main risk factors influencing their occurrence and persistence in food-producing environments and food'*, it was clarified that:

Accordingly, ToR 2 has been reformulated as (AQs):

- **AQ2a.** Among the antimicrobial-resistant bacteria and/or resistance genes contaminating food through the routes identified in this opinion, which are the ones of highest priority for public health?
- **AQ2b.** Which are factors that make a considerable contribution to their occurrence and persistence in food-producing environments and food?

For **ToR 3**, '*To review and, if possible, assess the impact of existing or new possible strategies and options to mitigate the risk of emergence' spread and food-borne transmission of the antimicrobial-resistant bacteria identified above*', it was clarified that:

- The opinion will include those strategies and control options that are already in place, as well as those not yet implemented.
- The opinion will include advantages and disadvantages of the mitigation options, as well as, if possible, a qualitative evaluation of their efficacy. Economic or environmental impacts will not be considered.
- The impact on the human exposure to ARB/ARGs resulting from these mitigation options will not be assessed.

Accordingly, ToR 3 has been reformulated as (**AQ**s):

- **AQ3a**. What are the possible strategies and options to mitigate the emergence and spread in the food-producing environment of the antimicrobial-resistant bacteria and resistance genes identified in this opinion?
- **AQ3b**. What are the advantages and disadvantages of implementing these mitigation measures?

For **ToR 4**, '*To identify data gaps influencing the assessment of the food chain-related AMR risks posed by the environment and provide recommendations to inform future EU research priorities on this topic', it was clarified that:

- Knowledge gaps and research needs for each food-producing environment considered by the opinion will be assessed.

Accordingly, ToR 4 has been reformulated as (AQs):

- **AQ4a**. Which are the knowledge gaps influencing the assessment of the role played by the environment in the emergence and spread of antimicrobial resistance through the food chain?
- **AQ4b**. Which future EU research priorities on this topic could be recommended?

1.3. International actions and reports on the role of the environment in antimicrobial resistance evolution, dissemination and transmission

The role of natural and farm environments in the emergence, selection, dissemination and ultimately transmission of AMR has received much less attention than selection and transmission within and between humans and animals and most reports and policy documents focus on the clinical perspective. However, over the last decade there has been an increased emphasis on the environment with the 2015 World Health Organization (WHO) AMR Action Plan (WHO, 2015) highlighting concerns around the impact of antimicrobials in the environment. Specific questions include how AMR circulates through the environment and how resistant organisms can be transmitted to humans through food and the environment. Environmental aspects received great attention also in the European Union (EU) One Health Action Plan Against Antimicrobial Resistance[7] published in 2017, with 'environment' being mentioned over 30 times, acknowledging the environment as an important contributor to the development and spread of AMR in humans and animals. In 2017 the United Nations (UN) published a report on Emerging Issues of Environmental Concern, highlighting the environmental dimension of AMR as one of the most pressing environmental issues of our time (UNEP, 2017). This report gives details of the phenomena that drive selection and dissemination of AMR in the environment, but

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[7] https://ec.europa.eu/health/sites/health/files/antimicrobial_resistance/docs/amr_2017_action-plan.pdf

without providing details in relation to food-borne exposure and transmission. A commitment to help tackle AMR was agreed at the 3rd United Nations Environment Assembly in Nairobi in 2017 (UNEA 3) as part of a resolution on environment and health (UNEP, 2018). In the same year the International AMR Forum was established supported by the Wellcome Trust in the UK, UK Science and Innovation, the US Centers for Disease Control and Prevention (CDC) and the Canadian government (Wellcome Trust, 2018a). A working group of approximately 40 specialists produced a scientific white paper entitled 'Initiatives for Addressing Antimicrobial Resistance in the Environment - Current Situation and Challenges' (Wellcome Trust, 2018b) and a symposium was held in Vancouver in April 2018. The white paper, published in 2018, focused on areas relevant to this report including human and animal contamination of the environment, and antimicrobials used as crop pesticides. The authors concluded that human and animal waste introduces ARB and antimicrobials into the environment, where selection for AMR may occur with some evidence of onwards transmission to humans. In 2019 the Interagency Coordination Group on AMR (IACG) published their final report which was presented to the UN Director General (WHO, 2019a). The report acknowledges the role of the environment and the risks to food and feed production: 'although evidence remains limited, concerns are also growing about the impact of AMR on the environment and natural ecosystems due to overuse and discharge of antimicrobials and resistant micro-organisms in manure and waste from health care facilities and pharmaceutical manufacturing, commercial livestock and plant production, and fish and seafood farming, a problem that may be fuelled by changes in the world's climate'. In March 2019, as one of the actions in the Action Plan, the European Commission adopted the European Union Strategic Approach to Pharmaceuticals in the Environment (PiE),[8] which focuses on actions to address the environmental implications of all phases of the lifecycle of (both human and veterinary) pharmaceuticals, from design and production through use to disposal. It includes some quite specific actions on AMR and identifies 'the links between the presence of antimicrobials in the environment and the development and spread of antimicrobial resistance' as a knowledge gap. A progress overview of the implementation was published in 2020.[9] The EU Strategic Approach to Pharmaceuticals acknowledges that whilst there is currently no clear link established between pharmaceuticals present in the environment and direct impacts on human health, the presence of antimicrobials (antibiotics and antifungals) may play a role in accelerating the development, maintenance and spread of resistant bacteria and fungi. The Strategic Approach also notes that there is limited monitoring of 'hotspot' locations, such as those affected by hospital effluents, and that even less is known about antimicrobial concentrations and AMR in soils. The latter may be a specific concern for food-producing environments where antimicrobials enter soils in livestock manures or through direct application to crops in the case of fungicides. The Organisation for Economic Co-operation and Development (OECD) report on pharmaceuticals and freshwater environments recommended that environmental risk assessment includes the risk potential of developing AMR (OECD, 2019). Also, the European Medicines Agency (EMA) has issued several considerations related to the approach to be followed to minimise environmental contamination with ARB and ARGs and to the assessment of the risk of antimicrobials for the environment (EMA/CVMP, 2021).

The European Green Deal[10] Farm to Fork Strategy[11] also highlights risks associated with AMR, with a 2030 target to reduce the sales of antimicrobials for farmed animals and in aquaculture by 50%.

Key outputs relating to AMR and food production include a WHO-funded systematic review and meta-analysis on AMU in livestock farming, and the link to AMR in animals and humans. The study informed the WHO guidelines on the use of antimicrobials in food-producing animals (Tang et al., 2017). Whilst the study did not specifically focus on the role of the environment, interventions that reduced AMU in food-producing animals were associated with a reduction in the presence of ARB in the animals. Some evidence suggests a similar association in humans, notably those with occupational exposure to the animals. There is far less evidence available for impacts on the general human population (Tang et al., 2017).

The Food and Agriculture Organization of the UN and the WHO produced a report in 2019 'Joint FAO/WHO Expert Meeting in collaboration with OIE on Foodborne Antimicrobial Resistance: Role of the Environment, Crops and Biocides' (FAO/WHO, 2019a) which concluded that 'there is clear scientific evidence that foods of plant origin may serve as vehicles of foodborne exposure to antimicrobial-

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[8] https://ec.europa.eu/environment/water/water-dangersub/pdf/strategic_approach_pharmaceuticals_env.PDF

[9] https://ec.europa.eu/environment/water/water-dangersub/pharmaceuticals.htm

[10] https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en

[11] https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal/actions-being-taken-eu/farm-fork_en

resistant bacteria'. It was also concluded that manure or organic wastes of human or animal origin have the potential to disseminate antimicrobial residues and ARB to the environment and that vegetables harvested from manured soils can carry an additional burden of AMR in the form of resistant enteric and/or environmental bacteria. Surface water and potentially wastewater used for irrigation were also reported as important sources of AMR and antimicrobials. Contamination of aquaculture products with bacteria carrying clinically important ARGs was also reported. The FAO/WHO (2019a) report also suggested that metal ions that have antimicrobial properties should be considered alongside antimicrobial residues in terms of probability of selection within agricultural environments. E.g. copper and zinc that are used in animal and plant production and which may co-select for AMR (Rensing et al., 2018). A more recent FAO Action Plan on Antimicrobial Resistance 2021–2025 (FAO, 2021) concluded that 'the unchecked spread of antimicrobial resistance (AMR) is on track to make drug-resistant infections the cause of the next pandemic. Agriculture is a source of antimicrobial resistant microorganisms, contributing to this problem'. The 2021–2025 FAO Action Plan also stated that 'contributing towards the goal of building resilience in the food and agriculture sectors by limiting the emergence and spread of AMR depends on controlling AMR effectively as a shared responsibility among farmers, herders, growers, fishers, prescribers and policy makers in food and agriculture – as well as other sectors'.

The WHO also recently published a technical brief to inform multi-sectoral national action plans regarding water, sanitation and hygiene (WASH) and wastewater management to prevent infections and reduce the spread or AMR (WHO, 2020), highlighting that AMR can be transmitted through water, sewage sludge and manure. The WHO report also highlighted the release of antimicrobials in faecal waste. Chemical substances shown to be of major concern for European Waters are listed in the priority substances (PS) list under the EU Water Framework Directive,[12] and Environmental Quality Standards (EQS) are set for these substances in a daughter directive (Environmental Quality Standards Directive[13]). Chemicals which have the profile of a priority substance owing to their toxicity, persistence and/or bioaccumulative properties, but for which monitoring evidence is lacking, may be put on the Surface Water Watch List (WL). Substances on this list should be monitored at a limited number of sites by EU Member States, to allow assessment of the level of exposure in the aquatic environment, and whether EU-wide EQS is warranted. Although pharmaceuticals are not included in the PS list yet,[14] the current WL includes a number of antimicrobials used to treat bacterial infection, including amoxicillin, ciprofloxacin, sulfamethoxazole and trimethoprim. The list also includes 10 azole antifungal agents used as human or veterinary medicines or to treat fungal infections in crops, which are of increasing concern due to emergence of azole resistance in human and animal fungal and yeast pathogens (Commission implementing Decision (EU) 2020/1161)[15] (see Section 3, Table 12 and Appendix A).

1.4. Antimicrobial resistance and known factors contributing to occurrence and persistence in food-producing animals, environments and food

Origins of AMR: Bacterial resistance to antibacterial compounds is an ancient phenomenon predating antimicrobial usage by humans by millions of years. Over millions of years many microorganisms have evolved the ability to produce chemicals (antibiotics) that kill or inhibit the growth of bacteria. In parallel, bacteria evolved various mechanisms to become resistant to these compounds. Thus, bacteria resistant to clinical antimicrobials have been found in cave systems isolated from the outside world and bacteria frozen within permafrost dating to 30,000 years BP carry clinically important ARGs (D'Costa et al., 2011). However, in the last 100 years there has been an increase in AMU in humans, animals and for plant production. This has exerted a much greater selective pressure for AMR in microbial communities associated with humans and animals, as well as natural

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[12] Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L 327, 22.12.2000, p. 1–73.

[13] Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. OJ L 226, 24.8.2013, p. 1–17.

[14] Under revision, considering several pharmaceuticals for possible inclusion.

[15] Commission Implementing Decision (EU) 2020/1161 of 4 August 2020 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council (notified under document number C(2020) 5205). OJ L 257, 6.8.2020, p. 32–35.

environments, through pharmaceutical manufacturing waste, excretion of antimicrobial residues by humans and animals and direct use of antimicrobials in food-producing environments (Figure 1).

Drivers of AMR in food-producing environments: prevalence and diversity of AMR in livestock-associated bacteria are a function of AMU and husbandry/biosecurity practices. The pressure for selection in the environment will depend on the concentrations of antimicrobials in animal faeces, in antimicrobial crop or aquaculture treatments, or in environmental sources entering food-production systems (e.g. antimicrobial contaminated water entering aquaculture systems) and how long these antimicrobial residues persist in the environment. Namely their persistence, and selective pressure is also influenced by their propensity to adsorb to soil or sediment particles where they may not be bioavailable. Antimicrobials (and other selective compounds) at bioavailable concentrations equal to or greater than the minimal selective concentration (MSC) are likely to contribute to AMR evolution in food-producing environments. It was thought that ARGs would impose a fitness cost, and thus at antimicrobial concentrations below the MSC-resistant bacteria carrying the ARG would be outcompeted by non-resistant bacteria. However, recent evidence suggests that several ARGs may impose a low fitness cost, be cost free (sometimes involving development of compensatory mechanisms), or in some cases confer a fitness benefit (Andersson and Hughes, 2010; Li et al., 2020; Kloos et al., 2021; Pietsch et al., 2021). Consequently, ARBs may persist within microbial communities for a duration related to the fitness cost of AMR and general ability to compete with other microorganisms, in some cases decreasing over time and in others persisting, or actually increasing, even in the absence of antimicrobial selection (Li et al., 2020; Perrin-Guyomard et al., 2020; Kloos et al., 2021). The interaction of environmental antimicrobial residues and microbial communities will not be dealt with in detail in this scientific opinion. However, it is important to note that the prevalence of AMR in food-producing environments, dissemination to the wider environment and therefore human exposure risk, is likely to be impacted by *in situ* selection where antimicrobial residues are above the MSC. The impact of low antimicrobial concentrations on AMR selection has been addressed in different studies (Andersson and Hughes, 2014), with some aspects concerning resistance development due to low antimicrobial concentrations (e.g. in feed and waste milk) covered by other published or on-going EFSA risk assessments (EFSA BIOHAZ Panel, 2017; EFSA BIOHAZ Panel, ongoing mandate on specific maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed; to be completed by 30 September 2021).[16]

The origins and transfer routes of AMR in food-producing environments are complex. Resistant bacteria and associated ARGs in food-producing environments can originate from indigenous environmental bacteria or be introduced by humans or livestock through animal and human waste streams such as manure, sewage sludge or faecally contaminated water. In addition, transmission between livestock and between wild animals and livestock will also affect AMR within livestock and food-producing environmental microbiomes.

In general, it has been suggested that the major risk factor for selection of AMR is related to antimicrobial use on the farms (EMA and EFSA, 2017; Jayarao et al., 2019). In line with the policy objective to reduce AMU in the food animal sector in the EU, the sales of antimicrobials declined by 34.6% in the EU in the period of 2011–2018 (EMA, 2020; More, 2020). However, reduction of AMU alone may therefore not be sufficient to control AMR because the environmental persistence and dissemination of ARB and ARGs is a major contributory factor. AMU correlates with AMR in human populations only when high levels of sanitation, and therefore, low levels of environmental transmission occur (Collignon et al., 2018). As opportunities for transmission increase the significance of AMU for AMR decreases. Environmental temperature, which is expected to rise, both in general and in terms of summer peaks as a result of climate change, appears to be another factor that influences AMR bacterial colonisation, possibly due to enhanced survival or multiplication of pathogens at higher environmental temperatures, and heat stress in animals making them more susceptible to infections (Kaba et al., 2020). Overall, the level of ARB colonisation is determined by a suite of factors including selection, transmission and climate conditions.

ARB and ARGs of concern: diverse species of bacteria are present in food; however, current surveillance focuses on a small number of priority pathogens and indicator organisms (see Section 1.6). When considering risk posed by AMR, important human pathogens are prioritised based on pathogen 'threat level' or more recently by ordering ARGs by hazard posed in terms of resistance to drugs of last resort. This is a logical approach, but it is also important to consider the complexity and fluidity of AMR in microbial populations. Susceptible pathogens can rapidly acquire ARGs, and priority

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[16] https://open.efsa.europa.eu/questions/EFSA-Q-2019-00221

ARGs can be readily mobilised from non-pathogenic bacterial hosts that are not monitored. It is therefore important to consider the implementation of culture independent surveillance methods as well as continuing to isolate important AMR clinical pathogens. Detecting the emergence of novel ARGs that may be transmitted through the food chain poses even greater challenges. The genomes of phenotypically resistant isolates of bacterial taxa that are the subject of surveillance can be sequenced and novel genes characterised using molecular genetic methods. However, genes present in non-pathogens or even in unculturable bacteria (most bacteria are not culturable) will not be detected. At microbial population level, metagenome-based sequencing approaches can be used to identify known ARGs and those with high similarity to known genes but even these methods will not detect novel genes if they are very different from known ARGs. Functional metagenomic approaches can be used to screen for novel ARGs, but they are expensive and time-consuming so are usually reserved for specific investigations, e.g. on the origin or source of particular genes that emerge in important clinical pathogens (Zhang et al., 2019a). A comprehensive SWOT analysis (strengths, weaknesses, opportunities and threats) of different uses of metagenomics for risk assessment of food-borne microorganisms, including metagenomics-based AMR monitoring was recently performed by the EFSA BIOHAZ Panel (2019a). From this analysis, it was concluded that among other applications, 'metagenomics has the potential to be used for risk assessment of food-borne pathogens, especially in relation to the identification and characterisation of non-culturable, difficult to culture or slow-growing microorganisms, the tracking of hazard-related genetic determinants and markers (e.g. AMR determinants, virulence determinants or markers linked to microbial behaviour) and the execution of risk assessments requiring the evaluation of complex microbial communities. Nevertheless, the impact of metagenomics on future risk assessment of food-borne pathogens will depend on the ability to overcome some current methodological constraints' (EFSA BIOHAZ Panel, 2019a).

For the detection of very rare resistance phenotypes in priority pathogens, enrichment culture using the target antimicrobial for selection is currently the most sensitive surveillance method, with subsequent whole genome sequencing (WGS) used to characterise the genetic basis of resistance. However, to better understand AMR selection in animal or environmental microbiomes, metagenomics or quantitative/real-time PCR may be more appropriate to determine changes in ARG prevalence within those microbial populations over time. Different methods measure different endpoints, so a combination of approaches will give the best overview of AMR in any given bacterial population.

In conclusion: ARB present in food will be related to the AMU regime in the food-producing system, the livestock management strategies (e.g. high- or low-stocking densities, housed or free-range animals, etc.), infection, animal waste management practices and farm hygiene control strategies and the use of other potentially selective or co-selective compounds such as heavy metals and biocides. The source and type of commercial feeds as well as the influence of airborne or waterborne pollution is also relevant. The type of food-producing environment, management strategies and physico-chemical interactions between antimicrobials and the environment will also affect the levels and types of AMR. The proximity to other food-producing systems and watercourses will also affect AMR dissemination. E.g. extensive grazing systems will have very different variables impacting AMR than intensive production systems. AMR in food will also be associated with processing methodologies and the probability of faecal/environmental contamination of the final food item. Certain types of food such as those eaten raw have been an area of particular concern and those likely to suffer from high levels of contamination, e.g. shellfish grown in estuarine or coastal regions impacted by human and livestock waste, should also be considered.

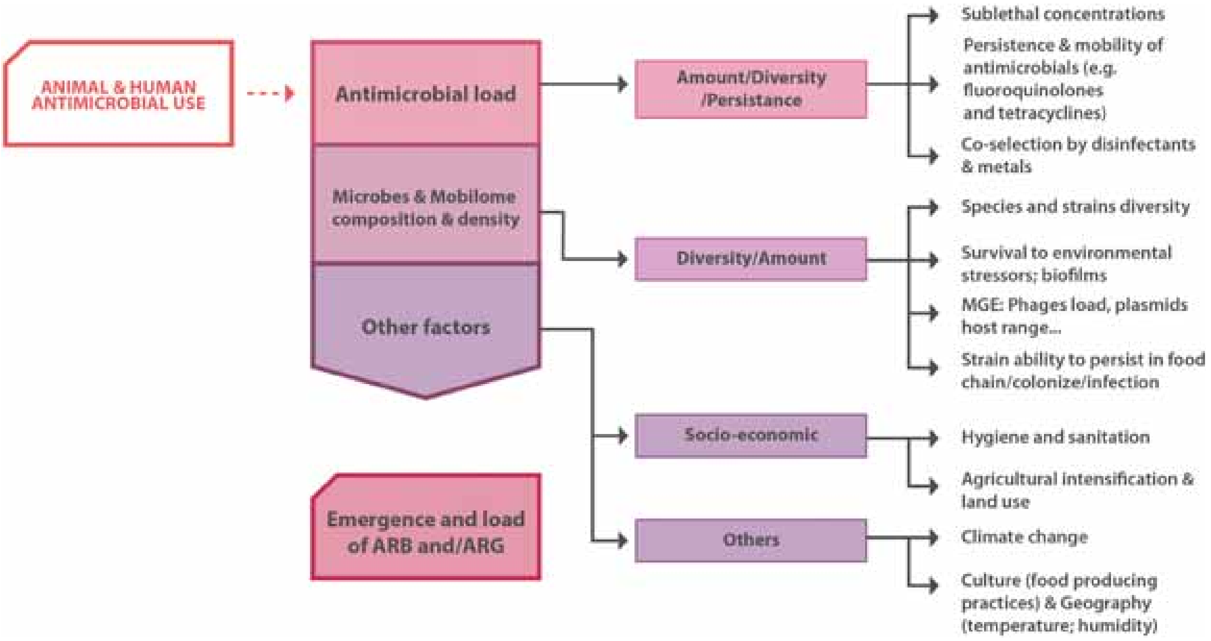


Figure 1: Examples of factors influencing the emergence and load of antimicrobial-resistant bacteria and/or resistance genes in food-producing environments

1.5. Antimicrobial-resistant bacteria, resistance genes and mobilome diversity

AMR surveillance, for both clinical and indicator organisms of animals and humans, focuses on a relatively small number of bacterial species, which differ widely in their life histories and mechanisms of acquiring resistance. However, AMR is not a characteristic specific to pathogens. It is present in all bacterial communities in environmental, animal and human microbiomes. Each gram of soil, sediment or faeces may contain approximately one billion bacteria belonging to thousands of species (Raynaud and Nunan, 2014) and AMR evolves within these complex communities in the presence of antimicrobial selection or other conditions which might favour acquisition of resistance (Figure 1).

Resistance can be intrinsic where a bacterium does not possess the specific target for an antimicrobial, or it is impermeable to the drug. Acquired resistance in previously susceptible bacteria is of greater concern and can occur through a variety of mechanisms including genome mutations, deletions, duplications or other genetic reorganisations. In addition, resistance can be acquired through acquisition of genes from other organisms through a process known as horizontal gene transfer (HGT) (Figure 2) (Martínez and Baquero, 2009). This is arguably of greatest public health concern and the acquisition of resistance to critical antimicrobials of last resort, as well as nearly all other antimicrobials, is often conferred by genes that are mobilised, including determinants for extended spectrum beta-lactamases (ESBLs) and carbapenemases, for example.

Genes are mobilised between bacteria through a wide range of mechanisms including direct transfer from bacterium to bacterium through a pilus or tube that connects cells in a process known as conjugation. The mobile genetic element (MGE) transferred is usually a DNA molecule known as a plasmid but there is a large diversity of MGEs that is ever expanding as we learn more about bacterial genetics (Partridge et al., 2018). There is a huge diversity in size and type of plasmids from small plasmids carrying a single gene to large plasmids comprising up to 10% of the size of a bacterial genome which might carry many different genes conferring adaptive traits. Some of these plasmids carry genes conferring resistance to a wide range of antimicrobials and are known as multi-drug resistance (MDR) plasmids. These plasmids allow a bacterium to gain multi-resistance in a single evolutionary step. Conjugation can occur between both closely related individuals of the same species but also, crucially, between completely unrelated bacteria. It is this reality, above all others, that illustrates the need to understand AMR at a microbial population level, rather than only focusing on pathogens. Further HGT mechanisms include transduction where viruses that infect bacteria, known as bacteriophage, can transfer genetic material between bacteria as part of their infection lifecycle. In some cases, DNA is mobilised from one cell to another where it integrates into the bacterial chromosome or an MGE, thereby conferring resistance. Another HGT mechanism is known as transformation, where naked DNA, released through cell lysis or actively excreted by some bacteria, is taken up by other bacteria.

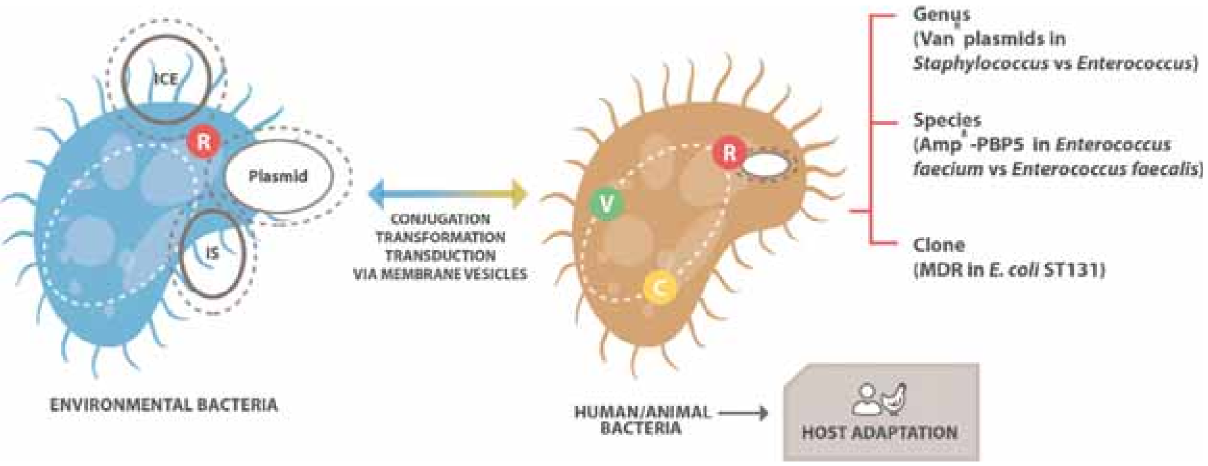
MGEs that can be transferred between bacteria are numerous, and many are located on plasmids in a fashion that has been described as a 'mosaic' or 'Russian doll' arrangement where smaller MGEs are located within larger MGEs in highly complex arrangements affording several levels of mobility. In addition to plasmids, which are generally circular DNA molecules present within cells, there are 'jumping' MGEs known as transposons that can move within and between genomes and plasmids. Other GEs known as integrons can integrate diverse mobile gene cassettes which confer resistance to nearly all known antimicrobial classes. It is not unusual to find resistance gene cassettes, integrated into integrons, situated on transposons embedded within plasmids or in integrative and conjugative elements (ICEs), for example (Figure 2). More recently, membrane vesicles are increasingly being shown to also play an important role in the dissemination of ARGs (Abe et al., 2020).

Co-selection is the term used to describe the phenomenon of enrichment (positive selection) of a resistance gene in the absence of the compound to which it is considered to confer resistance. This may occur through cross-resistance, where a gene confers resistance to more than one antimicrobial drug within the same class, antimicrobial drugs in different classes or to biocides and heavy metals. It may also occur through co-resistance, where genes are genetically linked on MGEs such as plasmids, whereby selection for one gene indirectly selects for all genes on that MGE. Both these processes are relatively common, which means that in general terms any antimicrobial may have the potential to select for a wide range of ARGs. This means that even antimicrobials only used in livestock, or older generations of antimicrobials not classed as critically important, have the potential to co-select for critically important ARGs associated with treatment failure in human infections. Co-selection can be demonstrated experimentally. In a study by Murray et al. (2019) ciprofloxacin exposure in a wastewater derived microbial community resulted in enrichment of aminoglycoside, beta-lactam, chloramphenicol, macrolide-lincosamide-streptogramin (MLS), sulfonamide, trimethoprim and vancomycin resistance genes compared with the control.

Because MGEs harbour genes conferring resistance to different antimicrobials, heavy metals and/or biocides, co-selection may occur, whereby an antimicrobial may indirectly select for resistance to unrelated antimicrobials or heavy metals and/or biocides (e.g. disinfectants/detergents). In this way, the selection for or maintenance of AMR can occur in the absence of antimicrobial drugs (in general or specific classes).

Bacterial virulence and host adaptation features are also part of the intricate network of several factors responsible for the evolution, transmission and maintenance of ARB. Interesting examples of host specificity are reported for *S. aureus* or *Salmonella* serovars, from animal or human host specific, to broad host range (Bäumler and Fang, 2013; Matuszewska et al., 2020).

Successful acquisition of ARGs may be influenced by how closely related acquired DNA molecules are (i.e. either plasmids or chromosomes), because codon usage, promoter binding sites and other transcriptional and translational motifs are likely to be similar. Those and other factors might justify genus, species or clone specificities favouring the acquisition and maintenance of certain MGEs carrying ARGs. E.g. there are fewer plasmids encoding vancomycin resistance in *Staphylococcus* spp. compared to *Enterococcus* spp., PBP5 variants conferring ampicillin resistance is amenable to conjugative transfer in *Enterococcus faecium* but not in *E. faecalis,* and the higher propensity of certain *E. coli* clones, such as ST131, to acquire specific CTX-M-encoding MDR IncF plasmids compared to others (Kondratyeva et al., 2020). However, it has also been reported that some plasmids can also be mobilised between distantly related bacterial species (Klümper et al., 2015). These examples again illustrate the complexity behind the emergence and success of ARG transfer and the difficulty in predicting future AMR threats (Figure 2).



Antimicrobial resistance genes (R) can be mobilised from an environmental microorganism by different mobile genetic elements (MGEs) (e.g. ICE-integrative conjugative element; IS-insertion sequence; plasmid) and transferred through conjugation, transduction, transformation or via membrane vesicles to another bacterial cell. Bacterial virulence (V) and host colonisation features (C) are also important factors for emergence and success of horizontally transferred AMR. Genus, species or clone specificities present in the food-producing environment may favour the acquisition and maintenance of certain MGE-encoding ARG. This transfer can also occur in the opposite direction: human and animal bacteria acting as donors of MGE carrying ARGs to environmental bacteria

Figure 2: Emergence and success of horizontally transferred antimicrobial resistance in the food-producing environment is multifactorial and complex

AMR is common in microbial populations. However, resistance is clinically important when acquired by human or animal pathogens or by commensal organisms that can act as ARG donors for pathogenic species. Prioritisation of pathogenic species has led to categorisation of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp. (ESKAPE pathogens, Rice, 2008) as of high public health importance, and in the context of food-producing environments, important food-borne pathogens include *Salmonella* spp. and *Campylobacter* spp. In addition, other Enterobacterales, including some *E. coli* strains, should be considered of high public health importance due to their ability to cause extra intestinal infections in humans (See 3.2.2 for detailed discussion). The WHO (2017) proposed a ranking of priority AMR pathogens including those mentioned previously. EMA/CVMP/CHMP (2020) listed examples of the most important AMR pathogens, including those of zoonotic relevance. Ranking ARGs in terms of public health importance is more challenging due to the extremely high diversity present in pathogens, commensals and environmental bacteria. ARGs can be ranked based on the class of antimicrobials they confer resistance to (e.g. WHO critically important antibiotics), whether the genes are known to be mobile, and/or associated with epidemic strains of human pathogens. However, it is still difficult to predict the identity of genes that may emerge in human pathogens in the future, so ranking systems should acknowledge this uncertainty. Further details of proposed ranking systems are discussed in 3.2.2.

1.6. Surveillance strategies for antimicrobial resistance in food-producing environments

In order to understand AMR in food production environments, a coordinated approach to surveillance in these complex sectors is required. Currently, most data available derive from either routine veterinary or food surveillance, which typically focuses on specific zoonotic pathogens and/or indicators from animal faeces or food. Further data are produced by one-off research projects, which typically use a wide range of non-standardised/non-harmonised methodologies and analysis pipelines to characterise AMR in faeces, soil, water, air or food. In the EU, the monitoring of AMR in zoonotic and commensal bacteria from food-producing animals and food thereof is performed yearly by the EU

Member States in a harmonised way in accordance with the EU legislation (Directive 2003/99/EC[17], Commission implementing Decision (EU) 2013/652[18] and 2020/1729[19]) (see Appendix A). This AMR monitoring is mandatory for *Salmonella* spp., *Campylobacter jejuni* and *E. coli* isolated from the major food-producing animal populations and derived meat (poultry including broilers, laying hens and fattening turkeys in even years, or fattening pigs and bovine animals under one year of age in uneven-numbered years). Additionally, the specific monitoring of extended-spectrum beta-lactamase (ESBL), AmpC beta-lactamase (AmpC) and carbapenemase-producing *Salmonella* spp. and *E. coli* is also performed. Some MSs also collect data on the prevalence, resistance and genetic diversity of Methicillin-resistant *Staphylococcus aureus* (MRSA). Those data, together with data on *Salmonella* spp. and *Campylobacter* spp. isolates from human cases of salmonellosis and campylobacteriosis, are published annually by EFSA and ECDC in the European Summary Reports (EUSR) on AMR in zoonotic and indicator bacteria from humans, animals and food (EFSA and ECDC, 2021a).

To gain a more complete understanding of AMR in food-producing environments firstly, the questions which surveillance strategies are designed to answer should be considered. This may include investigating AMR in complex bacterial communities to better understand the role that food production processes play in the emergence of AMR within animal gut and/or environmental microbiomes. Alternatively, the aim of surveillance may be to identify antimicrobial susceptibility in key pathogens across a wider study population to determine human exposure risk and trends. Huijbers et al. (2019) refer to these questions as ‘risk of evolution’ and ‘risk of transmission’, respectively, and characterise these and other wider environmental AMR surveillance questions with recommendations for surveillance targets and methodologies including culture independent methods, such as quantitative/ real-time PCR and metagenomic approaches. If surveillance aims to inform evolutionary changes in environmental microbial populations, then the antimicrobials themselves should also be considered for surveillance. The Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) recently funded 10 AMR surveillance networks, several of which consider surveillance in natural and farmed environments. One called ‘Towards Developing an International Environmental AMR Surveillance Strategy’ will produce a white paper in 2021 with recommendations for a range of approaches dependent on scope and available resources.

JPIAMR also funded a surveillance network focusing on ESBL-producing *E. coli* called the ‘Network for Enhancing Tricycle ESBL Surveillance Efficiency (NETESE)’ and will shortly publish an optimised protocol. If only one target must be chosen, ESBL-producing *E. coli* is a pragmatic choice. A further benefit of choosing *E. coli* as a One Health AMR surveillance target is that it has long been the subject of environmental surveillance such as in the EU Bathing Water Directive,[20] as well as an indicator of faecal contamination in food, e.g. for live bivalve molluscs and live echinoderms, tunicates and marine gastropods (Commission Regulation (EC) 2073/2005[21], amended with 2015/2285[22]). Also, the new European Regulation on minimum requirements for water reuse (EC 2020/741[23]) has selected *E. coli* for the routine monitoring of the performance of the wastewater treatment plants (WWTPs) (see Table 12 in Section 3.3.1.4, and Appendix A). Characterisation of the ESBL gene prevalence in aquatic *E. coli* has enabled human exposure estimates to be made at individual and population level (Leonard et al., 2015) suggesting that there are millions of annual exposure events where ESBL-producing *E coli* are ingested due to recreational coastal water exposure in England and Wales. Further work in the UK also suggests that high levels of bathing water exposure are associated with increased gut carriage of ESBL-producing *E. coli* (Leonard et al., 2018). Similar approaches are possible within food-producing

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[17] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. OJ L 325, 12.12.2003, p. 31–40.

[18] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32013D0652

[19] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020D1729&from=EN

[20] Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. OJ L 64, 4.3.2006, p. 37–51.

[21] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1–26.

[22] Commission Regulation (EU) 2015/2285 of 8 December 2015 amending Annex II to Regulation (EC) No 854/2004 of the European Parliament and of the Council laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption as regards certain requirements for live bivalve molluscs, echinoderms, tunicates and marine gastropods and Annex I to Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. OJ L 323, 9.12.2015, p. 2–4.

[23] Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 May 2020 on minimum requirements for water reuse. OJ L 177, 5.6.2020, p. 32–55.

environments and a recent study by Mughini-Gras et al. (2019) used genetic characterisation of ESBL genes in *E. coli*, combined with statistical modelling, to conclude that although most transmission of community-acquired ESBL-producing *E. coli* occurs between humans, the spread of ESBL-producing *E. coli* in human populations in the community is unlikely to be self-sustaining without transmission to and from non-human sources.

Recently, EFSA revised previous technical specifications on harmonised monitoring of AMR in zoonotic and indicator bacteria from food-producing animals and food and made suggestions for further AMR monitoring, also covering some aspects related to the environment (EFSA, 2019). These recommendations were considered for the revision of the current new EU legislation on harmonised monitoring of AMR in food-producing animals and derived food (Commission Implementing Decision (EU) 2020/1729). Among a number of other enhancements put forward, the technical specifications detailed proposals for specific AMR monitoring in the environment (shellfish), and a possible extension of AMR monitoring to aquaculture and seafood considering that in contrast to major livestock production systems, no widely adopted standardised AMR monitoring in aquaculture and seafood has been agreed. To obtain an overview of AMR in important aquatic animal/food/categories EU-wide baseline surveys were proposed.

The proposal presented in the technical specifications when regarding AMR in bacteria from the environment was the following: 'To carry out a baseline survey on AMR in bacteria from domestically produced shellfish that may address simultaneously consumer exposure via shellfish and environmental exposure of shellfish to resistant bacteria from, e.g. wastewater. It is envisaged that the detailed harmonised protocol of that specific baseline survey would be designed at a later stage, considering the most recent data, once a clear agreement to carry out such surveys had been reached. It is considered that additional interesting data on AMR in the environment might be available from testing bacteria gathered from the monitoring programs on bathing water quality within the framework of the EU 'Bathing Water' Directive 2006/7/EC, although these are not considered within the realm of this document'. With regard to a possible extension of AMR monitoring to aquaculture and seafood it was proposed 'To perform complementary cross-sectional/baseline surveys on AMR in bacteria from aquaculture and/or (imported) seafood, over the period of validity of the upcoming Commission Implementing Decision in 2021 onwards'.

1.7. Previous EFSA Opinions of interest to this Mandate

During recent years, EFSA has produced, alone or in collaboration with other European Agencies (ECDC, EMA), several reports and scientific opinions in which different aspects related to the public health risks of AMR, and specific ARB or ARGs in food and food-producing animals have been analysed. These have mainly covered: the extent to which food serves as a source for the acquisition, by humans, of ARB or bacteria-borne ARGs, to rank the identified risks and to identify potential control options for reducing exposure (EFSA, 2008a), 'the public health risks of bacterial strains producing extended-spectrum beta (β)-lactamases (ESBL) and/or AmpC β-lactamases (AmpC) in food and food-producing animals' (EFSA BIOHAZ Panel, 2011a,b), 'carbapenem resistance in food animal ecosystems' (EFSA BIOHAZ Panel, 2013a,b), 'measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union (EU) and the resulting impacts on food safety, taking into account the impact on public health and animal health and welfare-RONAFA' (EMA and EFSA, 2017), 'a list of outcome indicators to assist EU MSs in assessing their progress in reducing the use of antimicrobials and AMR in both humans and food-producing animals' (ECDC, EFSA and EMA, 2017a). All these reports/opinions contain relevant information for the current opinion, e.g. in some cases including the investigation of some aspects related to food-production environments; also general concerns and mitigation options to reduce AMR applicable for the sectors included in this, and consequently in the food-producing environments. Detailed information on these and other EFSA documents relevant for the present opinion can be found in Appendix B.

2. Data and methodologies

2.1. Data

Information was extracted from reports of international organisations (FAO, EC, ECDC, EFSA, EMA, OECD, OIE, Wellcome Trust, WHO) mentioned in Sections 1 and 2.2.1, European Legislation (see Appendix A) and the scientific literature.

2.2. Methodologies

2.2.1. Literature searches

A qualitative assessment of the role played by the environment in the emergence and spread of AMR through the food chain based on the available literature and expert knowledge was undertaken. Literature searches were broadened out using 'footnote chasing' (White et al., 1992) and supplemented by citation input by Working Group (WG) members and information about relevant publications provided by members of the EFSA Biological Hazards (BIOHAZ) Panel. The relevance of the records in providing information was assessed by screening the title, keywords and the abstract and based on the knowledge and expertise of the WG members.

In order to answer ToR1–ToR3, the relevant literature was reviewed. This review included international reports and EFSA Opinions, scientific review papers, book chapters, peer-review papers known by the experts or retrieved through non-systematic searches as well as current European Legislation. In addition, manual searching of the reference list of these documents was performed to identify additional relevant information. The focus was on European literature in relation to the types of ARB and ARG detected, but global literature was used to review generic aspects relating to AMR.

In addition to the generic searches mentioned above, the approach used to answer ToR2 included two steps:

1) The first step consisted in the identification of the ARB and ARG of public health priority.

   This was based on existing AMR hazard prioritisation approaches in scientific literature and reports from EU and international organisations (WHO, 2017, 2019a,b; EMA, 2020) as well as on further criteria defined in the framework of this opinion with the aim to refine the ARB/ARG of particular relevance in the context of the food chain. For ARB, criteria considered their pathogenic potential and their resistance profile. For ARG, they considered the resistance conferred and their location on mobile genetic elements. A more detailed explanation is provided in Section 3.2.2 and Table 6.

2) The second step consisted of the analysis of existing scientific evidence in relation to the selected priority ARB/ARG and their occurrence in the relevant environmental sources identified by ToR1. In particular, scientific literature, identified through a number of specific searches as well as additional scientific papers identified by WG experts, was screened to highlight evidence of the occurrence of priority ARB/ARG in environmental sources and of their possible transmission through food. Results are summarised in tabular form (Tables 7–11), with supporting information provided included in Appendix E. Specific search strings used for the different food sectors are listed in Appendix C.

In order to answer ToR4, the specific results, conclusions and uncertainties relating to the assessments carried out in ToRs 1–3 were reviewed and subjected to a 'brain-storming' exercise in order to confirm and refine the data gaps and consequent research needs that had been noted therein. Additional references were added in support of these aspects, where indicated, using bespoke searches in PubMed, Scopus and/or Google Scholar and focussing primarily on the most recent review articles.

2.2.2. Uncertainty analysis

The uncertainty in this Opinion was investigated in a qualitative manner following the procedure detailed in the EFSA guidance on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018a,b). The sources of the main uncertainties were identified and for each of these the nature or cause of the uncertainties was described by the experts. Expert judgement was used to estimate the individual impact of each of the uncertainties on the possible role played by the food-producing environment in the emergence and transfer of AMR and on the general conclusions (Appendix D, Table D.1).

3. Assessment

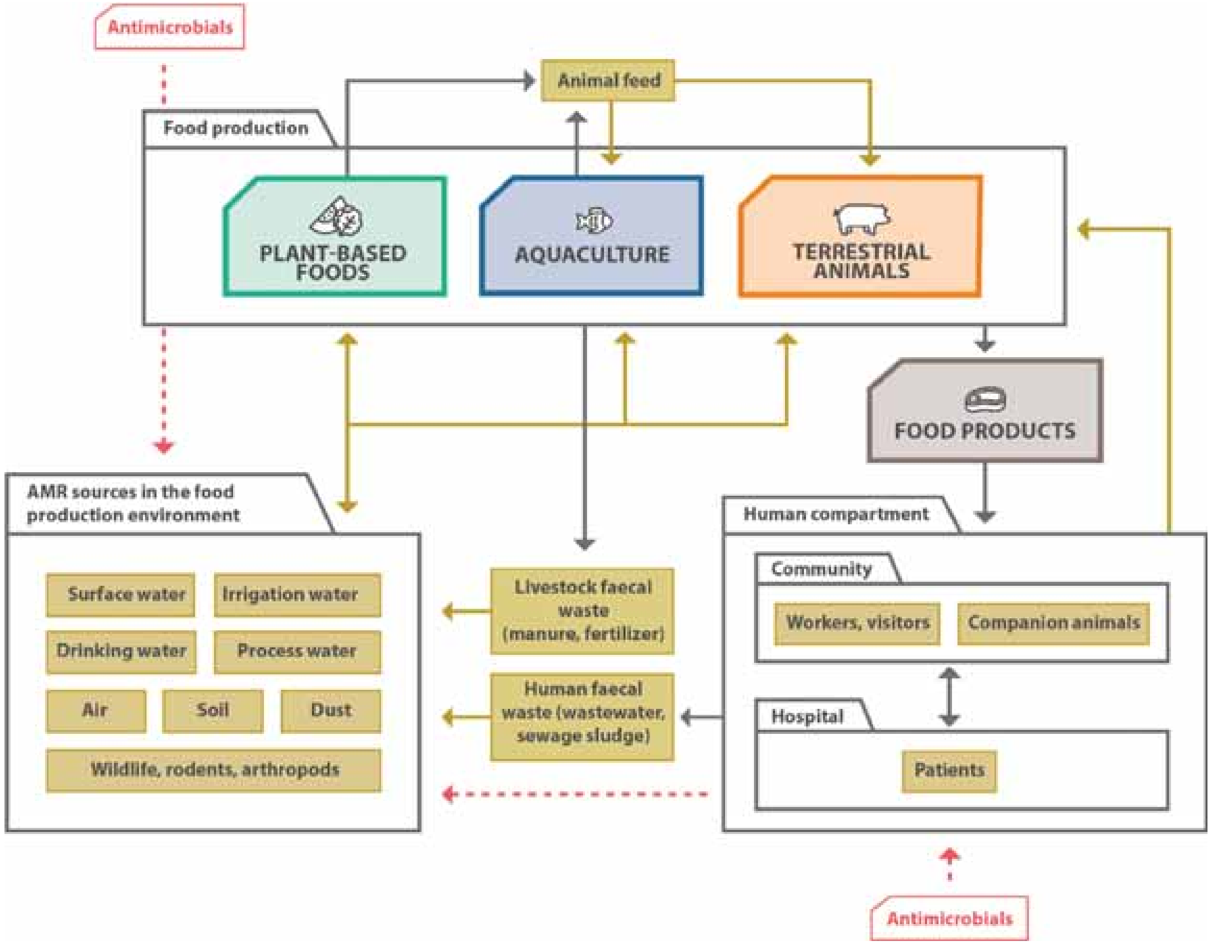
3.1. Environmental sources and transmission routes for antimicrobial resistance in different food production sectors

Food production sectors are linked with both humans and other possible sources of AMR in a cyclical manner, as shown in Figure 3. Through food production, ARB and ARGs can be transmitted between animal and plant-based food products and humans. Through effluent of wastewater treatment plants (i.e. treated wastewater and reclaimed water) and sewage sludge, human waste can lead to the contamination of surface water, soil and air. Water, soil and airborne dust in turn can act as transmission routes of AMR to contaminate the food production environment. Farm workers, visitors and companion animals can introduce AMR into food production systems. Other sources include contaminated production equipment, wildlife, rodents and arthropods. These sources can also contaminate animal feed used in food-animal production. Antimicrobials, heavy metals and biocides are also used in animal production and some of these may present a selection pressure for the development and transfer of AMR, as previously indicated.

The specific sources and transmission routes identified for each food-production sector environment will be graphically represented in specific sector maps (Figures 4–13) and described in detail in the following section and Appendix E. Where available, literature is presented that describes the presence of a specific transmission route in a food production sector, including both the presence of AMR in a particular source, and transmission from that source to the sector in question. However, often, literature on environmental transmission of AMR is absent. In that case, data on enteric pathogen transmission, or data on the presence of AMR in a particular source are used to support descriptions of possible transmission routes. A detailed overview on the ARBs and ARGs found in those sources is presented in Section 3.2 and Appendix F.

To harmonise the terms used in the sector maps presented in this section, major terms grouping different related terms were used. These terms are included in the Glossary.

The impact of the use of antimicrobials in agriculture on the development and spread of AMR has been extensively reviewed in a previous opinion (EMA and EFSA, 2017). The scope of the present Scientific Opinion is restricted to a critical review of current knowledge on the role of the environment for the introduction of AMR into different food production sectors.



Potential sources of AMR (resistant bacteria, both human pathogenic, zoonotic, commensal or environmentally associated and/or resistance genes) for the environments of different food-production sectors are shown as dark gold boxes, transmission routes as dark gold arrows. Black arrows depict the food production chain and the overlap between subcategories of humans (within humans there are more risk groups – patients provided as one example for such groups). Red arrows depict the usage of antimicrobial agents in food production and in humans and its effect on AMR. Definitions of terms used are given in the glossary

Figure 3: Environmental sources and transmission routes of AMR across food-production sectors

3.1.1. Plant-based food production sector

The EU is a major producer of fresh produce, crops and feed for food production animals. In 2019, the estimated harvested production in million tonnes of cereals and fresh vegetables was 299.3 and 60.9, respectively, together with the production of around 35.8 million tonnes of fruit (excluding grapes, strawberries and olives), 9.8 of olives and 22.3 of grapes generally used for olive oil and wine production (Eusrostat, accessed February 2021)[24,25] .

Production practices for plant-based foods vary considerably according to the agronomic needs of the particular product, the climate conditions at the point of production and the cultivation systems (e.g. open field, protected cultivation systems). Crops can be grown under conventional or alternative cultivation systems. Alternative production systems include soil or soil-less cultivation (e.g. hydroponics, aquaponics) and protected crops (greenhouses and crops grown under cover) among others (EFSA BIOHAZ Panel, 2013a,b). Greenhouses are generally used for growing high value products, but this is currently an expanding trend in many countries. Under mild winter climatic conditions, cold greenhouses and protected cultivations concentrate on vegetable productions belonging to the Solanaceae (tomato, pepper, eggplant) and Cucurbitaceae (melon, summer squash, watermelon, cucumber) families. These crops account for > 80% of the protected area in most Mediterranean countries (FAO, 2013). Crops grown under cover also include the use of low tunnels and mulching. These are used mainly for early production of several fruits such as cucumber, tomatoes and melons. Mulching is the covering of the soil surface with any material which separates the soil from the atmosphere. Mulching material can be either organic (e.g. crop residues such as straw) or inorganic (e.g. plastic film) (FAO, 2013).

Periodic outbreaks of food-borne disease due to consumption of contaminated fresh produce or sprouts are a cause for concern and serve as a reminder that vigilance with respect to microbiological safety is continuously required (EFSA BIOHAZ Panel, 2011a,b, 2013a,b; EFSA and ECDC, 2021b). Specifically for AMR, studies of the microbiome on fresh produce at retail have revealed many types of ARB and ARG of clinical concern, and the potential for HGT of determinants between different types of bacteria (see Section 3.2).

Intrinsic characteristics of the crops as well as applied agricultural practices determine the risk of contamination by AMR of environmental origin. Crops grown in the open environment are potentially exposed to numerous sources of ARB including wildlife, contaminated irrigation water and fertilisers of faecal origin such as manures and sewage sludge. In open systems, it is often difficult to associate the burden of AMR on product to a specific source as many factors intervene concurrently. Soilless culture that uses chemical nutrient solutions sufficient to meet the plants' demands will offer fewer opportunities for faecal contamination than, e.g. open field production with organic fertilisers (EFSA BIOHAZ Panel, 2014a). However, protected and open-field crops can share some risk factors such as the use of contaminated irrigation water and potential exposure to run-off. If the food type is a seed that is protected within a pod or shell it will be protected during growth in the field to a larger extent than leaves or roots (Cerqueira et al., 2019a). Root crops have close contact with soil, which may be amended with fertilisers of faecal origin. On the other hand, leafy vegetables that present a large surface to the environment can receive the most exposure, through irrigation, for example (Christou et al., 2019).

In general, the use of antibiotics as plant protection agents is not authorised in the EU, although some derogations are in place for certain member states in order to control specified plant pathogens with specific substances. Accordingly, we have not included AMU as a risk factor in the figures of the plant-based sector presented below.

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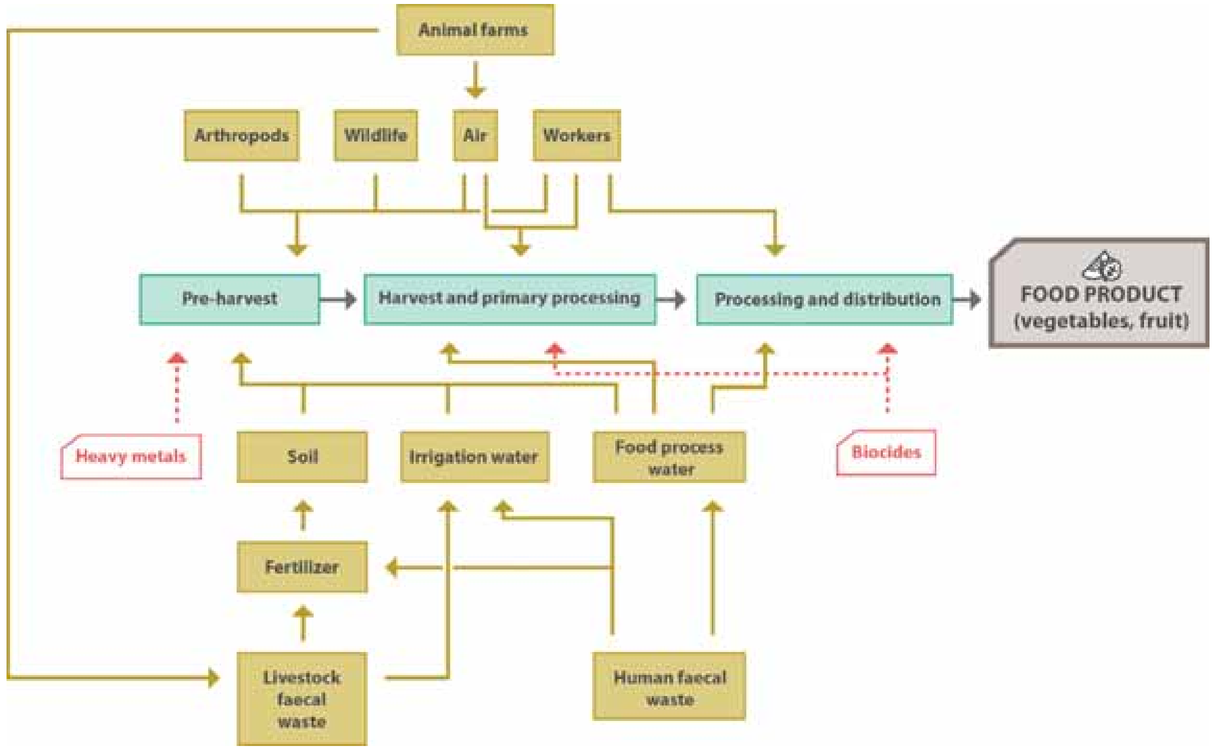
[24] Editorial note in the Eurostat reference cited: 'throughout this article, which deals with time periods when the United Kingdom was a Member State of the European Union, the acronym EU, however, refers to EU-27, the post-Brexit composition of the European Union as of 1 February 2020'.

[25] https://ec.europa.eu/eurostat/statistics-explained/index.php/Agricultural_production_-_crops (last accessed February 2021).



Potential sources of AMR (resistant bacteria, both human pathogenic, zoonotic, commensal or environmentally associated and/or resistance genes) for the food-producing environment are shown as dark gold boxes, transmission routes as dark gold arrows. AMR can either be introduced from these sources into the food chain, or AMR can also flow from the food production chain to these sources. Black arrows depict the flow of AMR along the food production chain (green boxes). Red arrows depict the usage of antimicrobial agents, biocides or of heavy metals (if applicable) in food production and its effect on AMR (selection of AMR). Human faecal waste includes treated and untreated wastewater as well as sewage sludge, and livestock animal waste includes fertilisers such as manure. Workers and visitors signify people with access to the production environment, for either professional or other reasons. Wildlife includes all animals with access to the production chain (such as birds, larger mammals) but excludes pests typically associated with food production. Rodents include vertebrate pests such as rats and mice. Arthropods include non-vertebrate pests such as flies, other insects and beetles. Companion animals are limited to those animals having access to the production environment. Definitions of terms used are given in the glossary.

Figure 4: Sources and transmission routes of AMR in plant-based food-producing environments

3.1.1.1. Field level AMR sources and transmission routes

Figure 4 summarises environmental sources of AMR and the route of introduction into production of plant-based foods. Soil that is used to grow fruits or vegetables can be fertilised using faecal material of human (sewage sludge, biosolids) or animal (manure) origin.

Manures, wastewater from farm buildings and yards, run-off from fields where fertilisers of faecal origin have been applied and sewage sludge (or municipal biosolids) contain ARB that are present in the digestive tracts of food-producing animals or people (Heuer et al., 2011; Lau et al., 2017; Glaize et al., 2020). They may also contain residues of antimicrobials or other potential co-selective chemical agents such as certain biocides or heavy metals (Sabourin et al., 2012; Van den Meersche et al., 2019).

The amount and types of microbial and chemical contaminants of concern in manure will vary according to the source (e.g. pigs, poultry, cattle) and the chemical inputs used in the production system; e.g. the types and amounts of antimicrobials or heavy metals used. Application of manure onto open fields can impact the soil microbiome locally by means of dissemination of ARB/ARGs, antimicrobials, biocides or heavy metals (Ye et al., 2007; Graham et al., 2009). The relative increase of AMR in manured soil in turn depends on the type of manure (Zhang et al., 2017). With respect to poultry litter, wet litter provides a greater abundance and diversity of bacteria than well-managed dry litter (Dumas et al., 2011). The same applies to pig and cattle slurry, as compared to farmyard manure, which includes bedding (Chee-Sanford et al., 2009; Davis et al., 2011; Heuer et al., 2011; Jechalke et al., 2013; Muurinen et al., 2017; Macedo et al., 2021). The minimum application rate of manure to be used on agricultural soils is determined by the Nitrate Directive (91/676/EEC)[26] as well as by national measures, and independent from its possible pathogen content. Guidelines for good practice[27] are described in more detail in Section 3.3.

As well as animal manure, sewage sludge is also used for soil fertilisation. The effect of sewage sludge application on the presence of resistant bacteria and resistance genes is less well established than for manure application and depends on the country, amendment rate and sludge treatment. There have been reports of a significant increase in the abundance of resistance genes in a Spanish study after amendment of soil with raw sludge (Urra et al., 2019), but also reports of negligible impact of repeated and single applications of treated sewage sludge on AMR in soil, although this finding may be influenced by the sensitivity of the test methods used (Rutgersson et al., 2020; Markowicz et al., 2021). The Sewage sludge Directive[28] determines practices (e.g. delays in application) to reduce potential health risks arising from the introduction of pathogens into soils, which are described in more detail in Section 3.3.

Dust containing antimicrobial residues or ARB can be emitted from livestock units or dried spread manure (Hamscher and Hartung et al., 2008; McEachran Andrew et al., 2015; Schulz et al., 2019) and can therefore represent an additional transmission route. The same holds true for soil particles that can be splashed up onto produce (Lee et al., 2019). Run-off from adjacent, fertilised or grazed fields may also move faecally contaminated soil particles to fresh produce (Barrios et al., 2020).

Irrigation with surface water, which may include treated or untreated wastewater, water that is otherwise polluted with faecal material and, to a lesser extent, with re-used (reclaimed) municipal wastewater effluent has the potential to contaminate fresh produce with ARB, as discussed in detail for pathogens (Uyttendaele et al., 2015; Benami et al., 2016; Christou et al., 2017; EFSA, 2017). Surface water can contain a considerable proportion of wastewater treatment plant effluent (e.g. secondary and tertiary treated wastewater) that is discharged upstream of the point of usage for irrigation (Keller et al., 2014). ARB/ARGs in wastewater can originate from both hospital and municipal wastewater, indicating that transfer of ARB of human origin from wastewater, sewage sludge or reclaimed water to fresh produce is a potential route of transmission back to humans. Pärnänen et al. (2019) found that AMU, environmental temperature and wastewater treatment plant size were important factors related with the presence of resistance in wastewater treatment plants.

The risk of faecal pollution of surface waters can be especially pronounced when sewage treatment plants and/or the capacity of manured or grazed land to retain faecal material are overwhelmed by

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[26] Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. OJ L 375, 31.12.1991, p. 1–8.

[27] Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene. OJ C 163, 23.5.2017, p. 1–40.

[28] Council Directive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. OJ L 181, 4.7.1986, p. 4–12.

heavy rain (Noyer et al., 2020). In many areas, climate changes that promote extreme precipitation events and consequently flooding are likely to complicate the challenge of minimising the risk of faecal pollution of the plant production environment (Boxall et al., 2009; Coffey et al., 2014; Gil et al., 2015).

Water reclamation and reuse for irrigation in agriculture are priority innovation practices. Water reuse is indicated by the European Commission (EC) as an important topic for the circular sustainable economy.[29] The agricultural re-use of municipal wastewater effluent will increase in importance in production areas that are becoming more arid due to climate extremes (Tram Vo et al., 2014). An Israeli study suggested that long-term irrigation with reclaimed water does not significantly increase ARG reservoirs in soil, but the possibility that additional clinically relevant ARGs not monitored in this study may be successfully transmitted from the wastewater to the soils could not be discounted (Negreanu et al., 2012). In the EU, the uses and minimum quality criteria for water reuse are laid down in Regulation (EU) 2020/741 (see Section 3.3.1.4, and Appendix A). Minimal criteria for bacterial contamination (in terms of concentrations of *E. coli*) are set out in this regulation, with required concentrations being below those that can be found in surface water affected by discharge of treated wastewater (Ouattara et al., 2013). The new Water Reuse Regulation (Regulation (EU) 2020/741) has been launched to boost the efficient, safe and cost-effective reuse of water for irrigation.

It is becoming increasingly evident that both avian and mammalian wildlife acquire AMR and are vectors for short and long-distance ((inter)continental) environmental transmission (Dolejska and Literak, 2019; Swift et al., 2019). The same probably holds true for arthropods (see Blaak et al., 2014, and section under terrestrial animals). Intrusion of wildlife onto farmland and faecal contamination of water used for irrigation by wildlife are likely to be important sources of contamination of fresh produce with enteric pathogens (Gutiérrez-Rodríguez and Adhikari, 2018); the same can be assumed for AMR (Plaza-Rodriguez et al., 2020).

Evidence for the above routes of introduction is collated in Table 1, including an expert assessment of the importance of the different routes based on the quality of the evidence.

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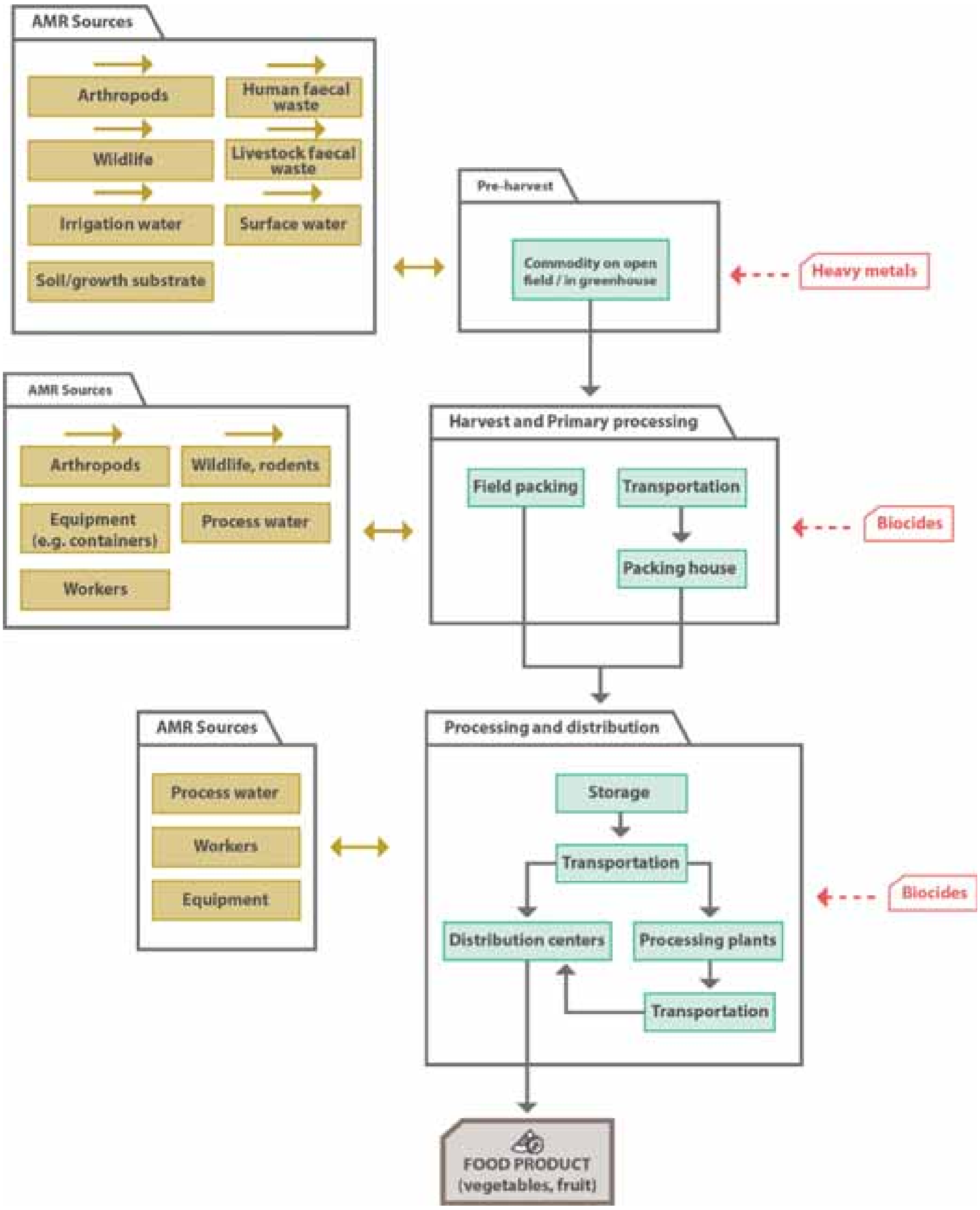
[29] https://ec.europa.eu/environment/strategy/circular-economy-action-plan_en#:~:text=The%20EU's%20transition%20to%20a,entire%20life%20cycle%20of%20products

3.1.1.2. AMR sources and transmission routes at harvest and handling



Potential sources and transmission routes of AMR (including antimicrobial-resistant bacteria (ARB) and resistance genes (ARGs)) for the food production environment are shown as dark gold boxes, transmission routes as dark gold arrows. AMR can either be introduced from these sources into the food chain, or AMR can also flow from the food production chain to these sources. Black arrows depict the flow of AMR along the food production chain (green boxes). Subcategories of the production chains are shown as folders. Red arrows depict the usage of antimicrobial agents (if applicable) and biocides and the presence and use of heavy metals in food production and its effect on AMR (selection of AMR). Human faecal waste includes treated and untreated wastewater as well as sewage sludge, and livestock animal waste includes fertilisers such as manure. Workers and visitors signify people with access to the production environment, for either professional or other reasons. Wildlife includes all animals with access to the production chain (such as birds, larger mammals) but excludes pests typically associated with food production. Rodents include vertebrate pests such as rats and mice. Arthropods include non-vertebrate pests such as flies, other insects and beetles. Companion animals are limited to those animals having access to the production environment. Definitions of terms used are given in the glossary

Figure 5: Detailed environmental sources and transmission routes of AMR–plant-based food production sector

The literature concerning the microbiological quality of fresh produce during production, at harvest and during handling and processing is focused on managing risks of contamination with enteric pathogens considering outbreaks of high public health relevance. Rinsing water used to clean produce in the field (Murray et al., 2017), contact with soil (Tien et al., 2017), harvesting equipment (Yang et al., 2012), reusable packing cases (Machado-Moreira et al., 2019) and humans (Julien-Javaux et al., 2019) are potential sources of microbial contamination at and following harvest. It is thus likely that the importance of pathways of AMR transmission to produce will vary with practice and hygiene status of postharvest water, equipment and workers handling the product. Information on transmission of enteric pathogens to produce would certainly underestimate levels of contamination with ARB, most of which will be commensals. Additionally, it should be taken into account that if monitoring of enteric pathogens and faecal indicator microorganisms, usually culture-based, is used to follow trends in AMR, this will potentially underestimate the occurrence of contamination with unculturable or viable but nonculturable (VBNC) ARB (Fleischmann et al., 2021).

3.1.1.3. AMR sources and transmission routes at processing and storage

Equipment and tools used during growing, harvesting and processing are well-known sources of contamination, as shown in Figure 5 and Table 1 summarising routes of entry of AMR into processing and storage of food of plant-based origin and need to be cleaned/sanitised accordingly (Marriott et al., 2018).

Any water used for processing or the preparation of ice used during storage and/or transport that is in direct contact with the edible part of the fresh produce must be of suitable microbiological quality (Machado-Moreira et al., 2019). Other potential sources at this stage include improperly cleaned/ sanitised contact surfaces, inadequate bulk storage facilities (poor air quality (dust), animal pests). Sanitisers and biocides in wash water have been hypothesised to also potentially select for AMR, but evidence for this occurring in food production and processing is lacking (Donaghy et al., 2019).

Arthropods can be also a source of AMR in this food-producing environment. Flies (e.g. *Musca domestica*) can carry and disseminate ARB including *Enterococcus* spp. in the food handling and preparation environment (Macovei and Zurek, 2006). Cockroaches may also be relevant vectors when the hygiene of food production environments is poorly controlled (Mpuchane et al., 2006).

During specific processes, bacterial growth, including that of pathogens and thus potentially of ARB, is favoured. This is, e.g. the case for sprouting (EFSA BIOHAZ Panel, 2011b; Karch et al., 2012), but also for fermentation (in which the pH range can, however, limit growth to a limited number of species).

Table 1: Stratification of environmental sources, transmission routes and risk factors of AMR –plant-based food production sector

AMR sources	Plant-based foods for animal use		Plant-based foods for human use				Supporting references	Comments
	Field crops					Covered		
Primary production	**Forage**	**Feed**	**Root**	**Leafy green**	**Aerial(b)**	**Vegetables, fruits and mushrooms**		
Irrigation water	++	++	++	++	++	+	Christou et al. (2017), Araújo et al. (2017), Gekenidis et al. (2018)	Aerial parts of the plant will be exposed in the case of overhead irrigation.
Animal waste(a)	++	++	++	++	+	+	Heuer et al. (2011)	Organic greenhouse production may use animal manure as a fertiliser
Human waste(a)	++	++	++	++	+	–	Bondarczuk et al. (2016)	Mandated delays between sludge application and harvest of crops destined for human consumption will attenuate risk. Situation with forages not as clear.
Workers/ visitors	–	–	–	–	–	+	Machado-Moreira et al. (2019)	
Wildlife	?/+	?/+	?/+	?/+	–	–	Swift et al. (2019)	
Air	?/+	?/+	?/–	?/+	?/+	?/–	Laube et al. (2014)	Contamination risk dependent on proximity to barns or other sources of airborne emissions
Growth substrate	–	–	–	–	–	+	Dróżdż et al. (2020)	Poultry manure can comprise part of the growth substrate and needs to be composted effectively.
Proximal animal farms	?/+	?/+	?/+	?/+	?/+	?/–	Holvoet et al. (2013)	Likelihood of contamination via runoff following manure application greater when livestock farm is nearby
Biocides	?/+	?/+	?/+	?/+	?/+	?/+	FAO/WHO (2018)	Although biocides could co-select for AMR, there is an absence of evidence that it occurs in production or processing environments

AMR sources	Plant-based foods for animal use		Plant-based foods for human use				Supporting references	Comments
	Field crops					Covered		
Primary production	**Forage**	**Feed**	**Root**	**Leafy green**	**Aerial$^{(b)}$**	**Vegetables, fruits and mushrooms**		
Heavy metals	+	+	+	+	+	+	Seiler and Berendonk (2012), EFSA FEEDAP Panel (2016), FAO/ WHO (2018)	Although heavy metals could co-select for AMR, there is an absence of evidence that it occurs in commercial production or processing environments. The EFSA FEEDAP Panel recommended ‘implementing a monitoring of copper pollution from agriculture in areas in which food-producing animals are fed, with particular attention to the potential development of microbial antibiotic resistance in the environment. The data would help to identify any area under risk’.
Harvest and handling	Forage	Feed	Root	Leafy green	Aerial	Vegetables & fruits		
Water	?/+	?/+	?/+	?/+	?/+	?/+		No literature available; relative risk based on expert opinion
Humans	?/+	?/+	?/+	?/+	?/+	?/+		No literature available; relative risk based on expert opinion
Wildlife	?/+	?/+	?/+	?/+	?/+	?/-		No literature available; relative risk based on expert opinion
Machinery, bins, totes	?/-	?/-	?/+	?/+	?/+	?/-		No literature available; relative risk based on expert opinion
Processing	Forage	Feed	Root	Leafy green	Aerial	Vegetables & fruits		
Water	?/+	?/+	?/+	?/+	?/+	?/+		No literature available; relative risk based on expert opinion
Humans	?/+	?/+	?/+	?/+	?/+	?/+		No literature available; relative risk based on expert opinion
Seed sprouting					++		EFSA BIOHAZ Panel (2011b)	Recommendations have been made for safe production, notably by protecting seed quality. These are intended for reducing risk from pathogens, not ARB.

Importance of the single sources is given from – (not important) to + (represents a source/factor of AMR, evidence for introduction existing for other bacteria), to ++ (important source/factor of AMR)). ?: specific information is missing.?/- No scientific evidence but presumably no source/factor, ?/+ No scientific evidence but presumably source/factor as AMR is present in the source and

introduction to the farm is possible. Importance is assigned based on the presence of supporting references and expert assessment, as scientific evidence on the relative attribution of AMR to the specific environmental sources is generally lacking. Dark gold: environmental sources, red: other selective pressures/risk factors.
(a): Sources of contamination (e.g. though manure or sludge).
(b): 'Aerial' food products include above ground vegetables, fruits and seeds (e.g. walnuts, olives, tomatoes, apples, etc.).

Uncertainties

There is limited information on the occurrence of AMR organisms (zoonotic, environmental or commensal) and of ARGs in plant-based foods as linked to specific sources, and a lack of prospective or intervention studies to provide scientific evidence to reduce uncertainty on the relative contribution of specific routes of transmission from environmental sources to plant-based foods. For more information see Appendix D, Table D.1.

3.1.2. Terrestrial animals production sector

About 77 million bovine animals, and 143 million pigs are present in the EU at any given time (Eurostat).[30,31] This results in 6.9 tonnes of veal and beef meat, 22.8 million tonnes of pig meat and 13.3 million tonnes of poultry meat (carcass weight) as well as 158.2 million tons of raw milk produced in 2019 (Eurostat).[32]

General factors that affect AMR in food products from terrestrial animals include antimicrobial use on the farms (which is not within the scope of this opinion), introduction from sources such as faecally contaminated water, soil or air/dust, animals with access to food production, workers and visitors and production equipment, as well as internal biosecurity and hygiene of production. Environmental elements such as watercourses or farm land and animal vectors can be contaminated by AMR originating from human waste and also by AMR originating from animal waste of the same or other types of farm animals, such as resulting from the application of animal manure.

In the following paragraphs, factors are described that contribute to the introduction of AMR to terrestrial animal food production systems and to the transfer of AMR between different food production systems, pre- and post-harvest (Figures 6–13). Preharvest data exist on the presence of AMR in several potential sources, but often not on actual transmission of AMR from such a source to a specific food production system. Nevertheless, these sources could potentially introduce AMR into food production environments. These sources are, therefore, described first and include contamination of water by animal and human faecal origin, and introduction with soil, air or dust as well as with wildlife. Post-harvest, information can often be only found for transmission of pathogens rather than transmission of ARB and ARG. Thereafter, the production chain for the poultry, pig, dairy and veal sectors and the factors that contribute to the introduction of AMR from environmental sources into these specific production systems are presented in a schematic way, with more detailed information presented in Appendix E. Where data on AMR is non-existent, examples for representative microbial pathogens, which often include strains with important or multiple resistances are considered.

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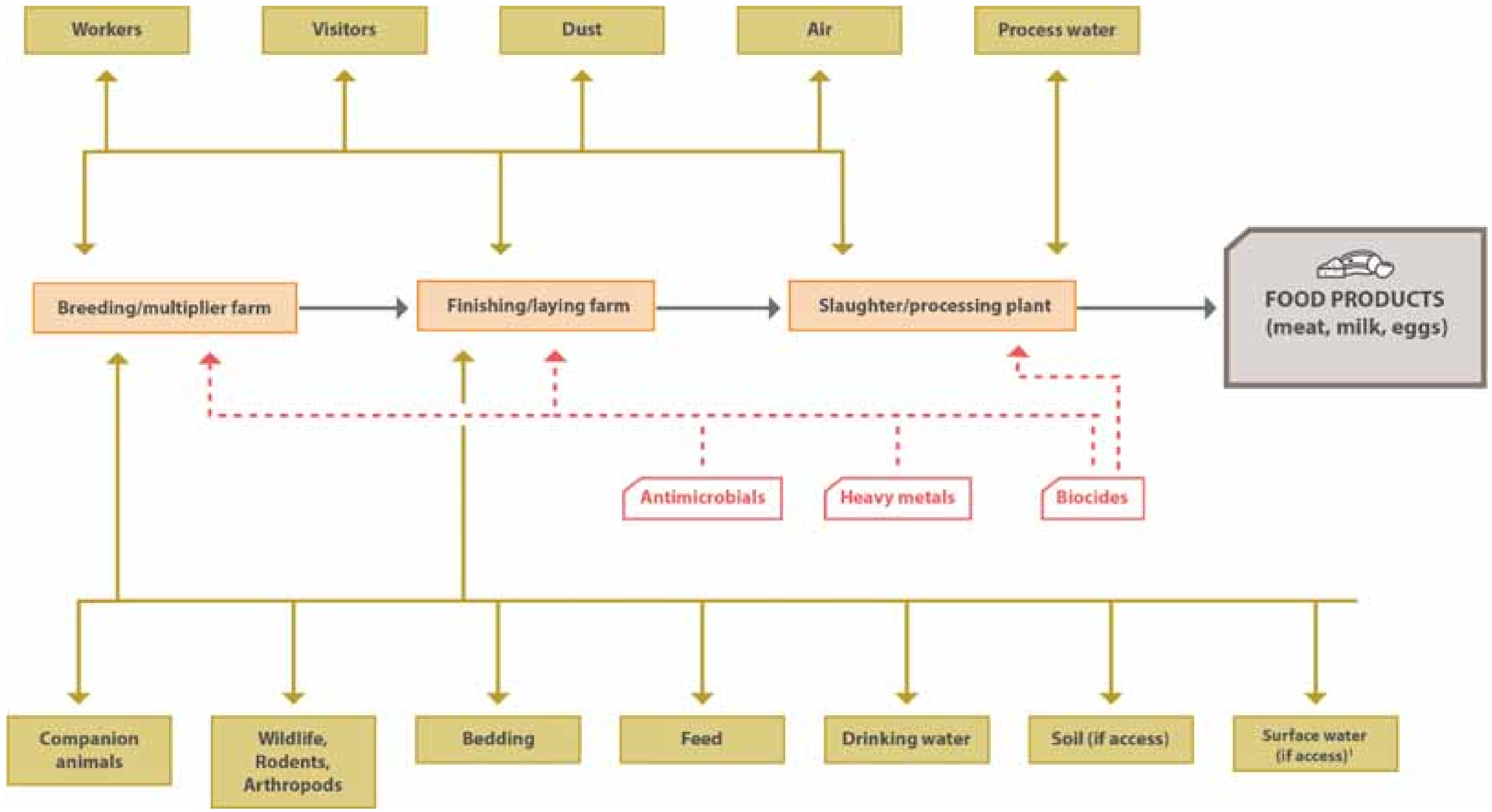
[30] Editorial note in the Eurostat references cited: 'throughout this article, which deals with time periods when the United Kingdom was a Member State of the European Union, the acronym EU, however, refers to EU-27, the post-Brexit composition of the European Union as of 1 February 2020'.

[31] https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agricultural_production_-_livestock_and_meat (last accessed February 2021).

[32] https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Milk_and_milk_product_statistics (last accessed February 2021).



Potential sources of AMR (resistant bacteria, both human pathogenic, zoonotic, commensal or environmentally associated and/or resistance genes) for the food production environment are shown as dark gold boxes, transmission routes as dark gold arrows. AMR can either be introduced from these sources into the food chain or AMR can also flow from the food production chain to these sources. Black arrows depict the flow of AMR along the food production chain (orange boxes). Red arrows depict the usage of antimicrobial agents and biocides in food production and its effect on AMR (selection of AMR). Workers and visitors signify people with access to the production environment, for either professional or other reasons. Wildlife includes all animals with access to the production chain (such as birds, larger mammals) but excludes pests typically associated with food production. Rodents include vertebrate pests such as rats and mice. Arthropods include non-vertebrate pests such as flies, other insects and beetles. Companion animals are limited to those animals having access to the production environment. Surface water and soil are only relevant if the food animals have access to it, e.g. during free range production. Definitions of terms used are given in the glossary

Figure 6: Environmental sources and transmission routes of AMR - terrestrial animal food production sector

3.1.2.1. Preharvest AMR sources and transmission routes

Additional information is included in Appendix E.

Soil, bedding and surface water

As discussed in Section 3.1.1, the application of animal manure as a fertiliser leads to dissemination of antimicrobial residues, ARB and ARGs of animal origin to soil. In addition, sewage sludge application can also introduce AMR into soils. From soil, ARB and ARGs can potentially enter different production systems, e.g. if dairy cows graze on manure-fertilised grasslands, or through dust particles originating from soil. This transmission is not limited to within-sector transmission but can occur between sectors. Furthermore, ARB from manure can further spread to surrounding water courses as ARB, ARGs and antimicrobials can be found in waterways after manure fertilisation followed by agricultural run-off events (Larouche et al., 2020).

There is little information on the relevance of manure for introduction of AMR to livestock farming systems, in contrast to the effects of manure fertilisation on the occurrence of ARB and ARGs in fertilised soils (Heuer et al., 2011).

If farm animals are exposed to waterways that are faecally contaminated, e.g. in free-range systems, exposure to ARB and ARGs that originated from another animal sector can occur (Moore et al., 2010), but there is no evidence of the impact of this at the EU level.

Bedding material contaminated with faecal matter might be also a potential source of AMR. In species in which bedding is frequently used, particularly cattle, some studies have stressed the role of bedding as a vector where ARB from faeces persists in large numbers and becomes source of contamination for animals which come into contact with this ARB-contaminated bedding (Yang et al., 2006; Subbiah et al., 2012; Astorga et al., 2019).

Air and dust

With respect to AMR in air and dust samples, research has mainly focused on the presence of ARB and/or ARGs within animal housing and in the nearby environment (Schmid et al., 2013; de Rooij et al., 2019; Hordijk et al., 2019; Luiken et al., 2020). Information on the introduction of airborne AMR with air or dust into a production system is sparse. Depending on the nature of the bacteria, viable bacteria might be dispersed for considerable distances. In particular, *Legionella* spp. has been shown to cause infections at distances of up to 12 km (Walser et al., 2014).

Different studies have isolated ARB in air or dust in pig facilities (Gibbs et al., 2004; Novais et al., 2013). The presence of ARB and MDR isolates suspended in air or dust can facilitate the spread and persistence of MDR strains within farms. In addition, the detection of ARB in air or dust from cleaned facilities (Braga et al., 2013; Novais et al., 2013) demonstrates the risk of transmission to subsequent batches of animals. ARB was also shown to disseminate for at least 150 m downwind of a pig farm (Gibbs et al., 2006).

A Spanish study observed that similar ARBs were found in the air inside and around dairy farms, and therefore concluded that emissions from the farm may act as a source of dissemination of ARB. Air sources have been investigated and it is possible for air to circulate inside and outside of the farm and transport resistant organisms to the surroundings (Navajas-Benito et al., 2017). In dust samples obtained from dairy farms with an electrostatic dust collector ARG were detected, however, as different ESBL genes were detected in dust than in the animals, exposure to air/dust might not represent the dominant transmission route for ESBL bacteria on dairy farms (Hordijk et al., 2019).

The negative pressure created by air extraction in poultry houses increases the risk of introducing airborne particles and flies (Geden et al., 1999; Silbergeld et al., 2008). ARB can be found in air within and around poultry houses including during the empty inter-crop period, albeit at lower levels than during the life of the flock (Brooks et al., 2010) and can disseminate organisms over a wide area, particularly where powerful extractor fans are used. Poultry house manure, litter or dust includes a complex matrix of faecal bacteria, nutrients for microbial growth such as undigested feed, and feed additives including antimicrobial agents, biocides and heavy metals (Deng et al., 2020). Survival of ARB in poultry house dust can be very prolonged, so maintenance work to poultry houses carried out many years after the original contamination can release viable bacteria (Schulz et al., 2016). Laube et al. (2014) investigated the potential emission and transmission of ESBL/AmpC-producing bacteria from broiler fattening farms, and their results suggested that these bacteria can spread from these farms via extracted stale air from the poultry houses, as well as in faecal waste and wastewater. *E. coli* and *S. aureus* of poultry origin were found to be above background levels on the leaves of trees 35 m, but not 120 m, from occupied poultry houses (Theofel et al., 2020). Dust contaminated by ARB is also created after spreading of poultry litter and manure on land, with the resulting small soil particles travelling longer distances than regular soil in windy conditions (Münch et al., 2020; Thiel et al., 2020), similar to the dust-borne spread of MDR monophasic *Salmonella* Typhimurium from outdoor pig ranges to enclosed poultry farms observed in UK after prolonged dry weather (APHA/Defra, 2019, 2020).

As an indication of animal-to-animal transmission, dust samples taken from pig barns have been shown to contain MRSA genotypes which were shared between dairy cow milk samples, humans and other pig farms in mixed farming settings (Locatelli et al., 2017). Similarly, in a study performed in Bavaria in Germany, dust samples obtained from mixed farms with dairy cows and beef were significantly more likely to contain ESBL-producing *E. coli* than samples from beef cattle farms (Schmid et al., 2013). Higher ESBL presence might also be related to higher AMU in the mixed farms. In any case, selection within the farm environment may lead to an increase of resistant organisms, which will likely recirculate.

While there are no direct data on air as a transmission route to animals, several studies showed that the air within pig premises may be a source of occupational transmission of ARB (Bos et al., 2016; Angen et al., 2017; Song et al., 2021) so transfer between animals through air is also likely. In addition, the scientific literature suggests that high-density animal areas increase the risk of carriage of LA-MRSA in surrounding residential areas, stressing the potential microbial air pollution from livestock farms (Feingold et al., 2012; Casey et al., 2013; Carrel et al., 2014; de Rooij et al., 2019).

Wildlife, rodents and arthropods

Wildlife, arthropods and rodents could be a source and transmission route of AMR threats impacting particularly the environment of primary production of the different terrestrial animal production food sectors.

Wildlife are attracted to farms because of the warmth/shelter, spilled feed, potential for harbourage and the smell of manure and mortalities.

In principle, all types of wildlife have been shown to be carriers of ARB, mostly acquired from human or livestock environments (Vittecoq et al., 2016; Darwich et al., 2019; Dolejska, 2020; Ramey and Ahlstrom, 2020). ARB are frequently found in wild mammals and birds although the direction of transmission between wildlife and livestock is usually unclear (Arnold et al., 2016).

Migratory birds are a particular threat and can transport ARB and ARGs internationally (Cao et al., 2020; Franklin et al., 2020; Lin et al., 2020). Wild birds, rodents (Jahan et al., 2020) and insects are particularly relevant potential sources of ARB if not properly controlled, as they are more likely to be able to access housing and feed or bedding stores, but the exterior of farm buildings or range areas of outdoor-access units can also be contaminated by the faeces of foxes, badgers or feral animals, as well as companion animals. In turn, insectivorous wildlife species, as well as farm animals, are likely to acquire ARB from flies (Royden et al., 2016; Nowakiewicz et al., 2020). However, despite numerous studies, there is no conclusive evidence that demonstrates transmission of ARB to food animals (Nielsen et al., 2018). An example for indirect evidence/association is the reported changes in ESBL gene families and the resistance phenotype of cefotaxime resistant *E. coli* isolates obtained from German broiler, pig, cattle and beef cattle farms when waterfowl were present within 1 km, as compared to farms that did not have waterfowl present (Hille et al., 2018a).

Other wildlife species might act as natural reservoirs for more specific resistant clones e.g. hedgehogs which seem to be a natural reservoir for *S. aureus* with *mecC* encoded methicillin resistance (Rasmussen et al., 2019). The presence of ARB has also been demonstrated in wild boar (Bonardi et al., 2019; Torres et al., 2020) and other animals which surround pig farms (Allen et al., 2011).

All in all, the limited understanding of the wildlife intestinal microbiome may limit efforts to assess AMR dissemination, as the likelihood of acquiring a certain species within the microbiome by wildlife species and the capacity to transmit it locally or at long-distance is relatively unknown. Furthermore, most studies rely on common commensal species and AMR determinants and on culture methods, but the spread of ARGs might be more complex.

Insects and flies can potentially be vectors of ARB and ARGs (Zurek and Ghosh, 2014). Flies are attracted to 'dead bins' and manure, particularly in droppings pits or manure stores, and have been shown to carry a multitude of ARB (Fukuda et al., 2019; Poudel et al., 2019) and to remain contaminated through various stages of metamorphosis (Maleki-Ravasan et al., 2020). In a German study, a lower prevalence of cefotaxime-resistant *E. coli* was found on beef cattle farms that employed fly control using traps – an indication that ARB can enter beef cattle farms with flies (Hille et al., 2017).

Companion animals and mixed farms

Companion animals could be a potential source of ARB and ARG, particularly in the primary production of food-producing animals. Although to our knowledge no data are available in relation to dogs and cats sampled on farms, they are often owned by farm managers. Companion animals have been observed entering range areas and empty poultry houses/service areas (Baede et al., 2017).

In mixed farms and in farms in which co-grazing occurs, farm animals may be also potential sources of different bacteria ARB in the food-producing environment, as they can be a reservoir for different pathogens (e.g. nearby cattle could be a potential reservoir of *Campylobacter* for commercial broilers, and pigs can be the source of MRSA in cattle reared on the same farm) (Hansen et al., 2019; Frosth et al., 2020).

Feed

Information about the presence of ARB and potential transmission of mobile genetic elements and associated ARGs in pig feed mills is scarce (Molla et al., 2010; Burns et al., 2015). Feed contamination has been primarily identified for pathogens rather than for ARB or ARGs; however, the pathogens identified can include resistant clones such as MDR *S.* Typhimurium, which can contaminate grain which is grown or stored on pig or cattle farms because of the activity of wild birds and rodents (Davies and Wales, 2013). Industrial compound feed has been identified as the feed group with the highest risk for contamination by *Salmonella* (EFSA, 2008b). Furthermore, forage or haylage can be faecally contaminated (FAO/WHO, 2019a), including by wild animals (Surette and Wright, 2017).

Heat treatment such as during pelleting reduces the bacterial load and the risk of the presence of pathogenic bacteria (Torres et al., 2011) and consequently reduces the risk of AMR. However, in order to minimise costs and heat damage to the nutritional quality of feed, the heat treatment only achieves a reduction in bacteria but not complete elimination (Cox et al., 1983). Feed processing practices can therefore result in the presence of pathogens and ARB in feed (Sapkota et al., 2007; EFSA, 2008b).

Heat-treated feed can be readily re-contaminated during cooling, transportation, storage and finally, on farm. Dust collected from coolers has higher contamination than dust from the other mill locations due to the increased moisture level inside the cooler which creates favourable conditions for growth of bacteria (Vukmirovic et al., 2017). Most feed mills have continuous or intermittent problems with stored-product insects and in a study in the US, 18% of 298 live insects from nine insect species collected from six feed mills yielded AMR *Enterococcus* spp. at levels ranging from 2×10^1 to 1.3×10^5 per insect (Larson et al., 2008).

International dissemination of bacteria is possible via trade in animal feed ingredients (Wierup, 2017; Fraiture et al., 2020).

Drinking water

Depending on the quality and origin, drinking water can be a source of entry of ARB into animal farms (O'Dwyer et al., 2018; Tanner et al., 2019), especially if drinking water is drawn from local surface water or taken from a private well (O'Dwyer et al., 2018), or if drinking water is not enclosed, with access for wild animals.

A Swedish study on dairy farms found that environmental samples (feed and water troughs in calving areas) collected from farms with high levels of quinolone resistant *E. coli*, showed greater contamination with these organisms than other sample types. Water and feed contamination therefore appeared to play a role in the dissemination of these organisms within the farms (Duse et al., 2016). The frequency of water trough disinfection was negatively correlated with the prevalence of cefotaxime-resistant bacteria in calves in a study in the US, suggesting transmission of ARB between animals through contaminated drinking water troughs (Markland et al., 2019).

Workers, visitors

Farmers, workers and veterinarians have a relatively close contact with farm animals and inadequate hygiene precautions can lead to the spread bacteria between animals (Williams, 1980). Evidence of humans as a potential primary source of ARB for farm animals, rather than acting as a vector for environmental contamination, is sparse, speculative and circumstantial, involving organisms such as LA-MRSA (Monecke et al., 2013; Park and Ronholm, 2021). However, other MRSA types such as CA and HA-MRSA strains have also been found among cattle in Europe (Schnitt and Tenhagen, 2019), suggesting that these strains have been introduced into the food production system from humans. Highly ciprofloxacin resistant *Salmonella* Kentucky was introduced to French turkey farms by a worker who had returned from a holiday in Africa (Guillon et al., 2013). *Salmonella* has also been introduced to cattle farms indirectly from human infection, and consequent environmental contamination after foreign travel (Johnston et al., 1981).

Other potential AMR sources

Waste milk (milk that cannot be marketed for human consumption, e.g. from cows treated with antimicrobials) may be used as feed in different groups of calves that may or may not join the dairy herd, e.g. veal or beef calves (EFSA BIOHAZ Panel, 2017). Feeding waste milk to veal calves at an early stage might be a source of AMR and a potential source of antimicrobial residues that can impact the microbiota at the development stage, and can contaminate the environment after disposal (EFSA BIOHAZ Panel, 2017). In addition, bacteria from the skin and intestinal microbiota or other environmental sources at the farm (e.g. from bedding, equipment or other sources) might be sources of contamination of milk for dairy calves through contamination of the udder.

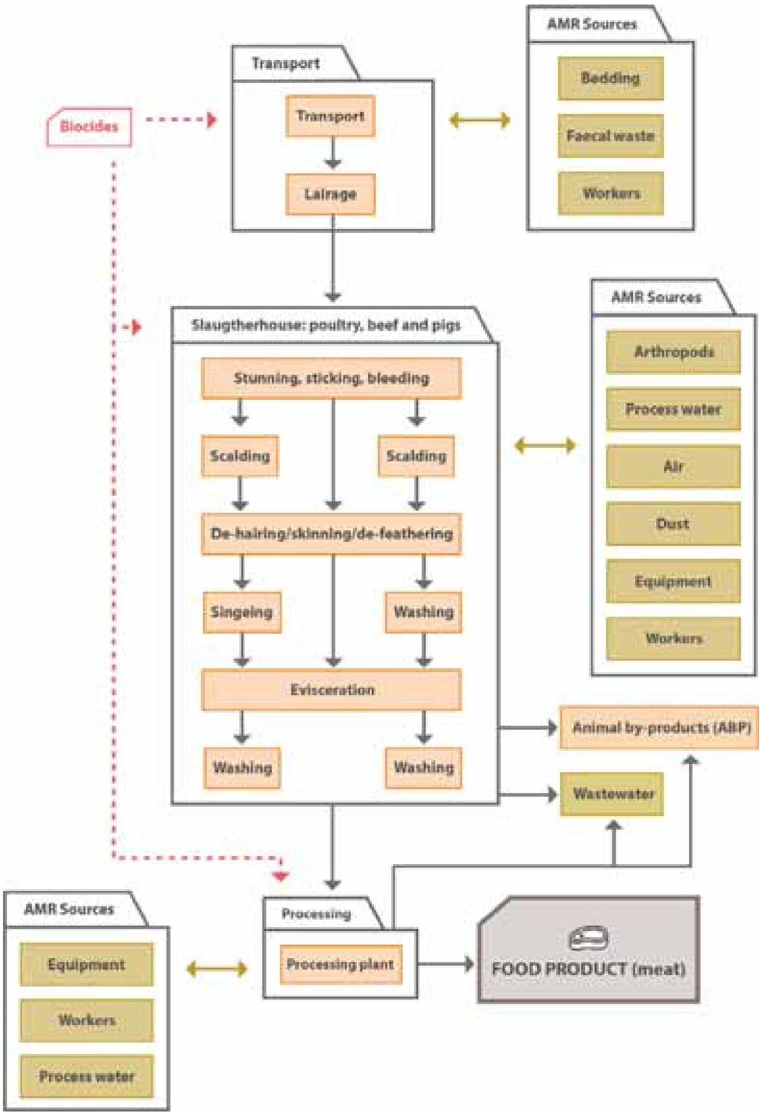
Indirect evidence for the role of environmental sources

Some general evidence on the role of external sources for AMR introduction comes from risk factor analyses. Analysis of management factors for the occurrence of cephalosporin-resistant *E. coli* in UK turkey flocks showed that presence of a watercourse or pig farms nearby were of particular importance (Taylor et al., 2016). Fluoroquinolone resistant *E. coli* isolates with similar resistance mechanism patterns were detected from pigs, wildlife and broiler flocks in Norway, despite minimal national usage in food animals. Phylogenetic analysis indicated close evolutionary relationships between some isolates from different sources, suggesting dissemination of strains between species (Kaspersen et al., 2020a).

While there are few studies directly addressing transmission from rodents into poultry farming, risk factors for occurrence of ciprofloxacin resistance in *E. coli* from turkey fattening flocks in the UK included evidence of mice as a source of these bacteria on the farm (Jones et al., 2013).

Similarly, analysis of management factors showed that for *Campylobacter* spp., including MDR strains, which are the most common in food animal production, on-farm hygiene, cleaning and disinfection between batches of birds and wildlife control are of greatest significance for poultry farms (EFSA, 2019).

3.1.2.2. Post-harvest AMR sources and transmission routes



Potential sources of AMR (resistant bacteria, both human pathogenic, zoonotic, commensal or environmentally associated and/or resistance genes) for the food production environment are shown as dark gold boxes, and transmission routes as dark gold arrows. AMR can either be introduced from these sources into the food chain, or AMR can also flow from the food production chain to these sources. Black arrows depict the flow of AMR along the food production chain (orange boxes). Subcategories of the production chains are shown as folders. Red arrows depict the usage of biocides in post-production and its effect on AMR (selection of AMR). Workers and visitors signify people with access to the production environment, for either professional or other reasons. Rodents include vertebrate pests such as rats and mice. Arthropods include non-vertebrate pests such as flies, other insects and beetles. Run-off signifies faecal contamination of adjacent surface waters when animal faecal matter is washed off the post-production environment with rain. Wastewater represents e.g. water used to clean the production or slaughterhouse environment that is then discharged into the canalisation or treated on-site before release to surface water. Definitions of terms used are given in the glossary.

Figure 7: Detailed environmental sources and transmission routes of AMR– pork, beef and poultry processing

Transport

Transport could be a transmission route for contamination by animals from different farms, as trucks visit multiple farms, sometimes mixing animals from different origins. Studies on *Salmonella* and *Campylobacter* have demonstrated the presence of resistant strains inside trucks (Gebreyes et al., 2004; Quintana-Hayashi and Thakur, 2012), even in samples collected before loading the animals, presumably linked to inefficiency of cleaning protocols (Mannion et al., 2007). Transport vehicles, modules and crates can spread pathogen contamination between the abattoir and poultry farms and between farms, particularly where partial sequential depopulation (thinning) is practiced (Buess et al., 2019; Rasschaert et al., 2020).

Slaughterhouse

Lairage

Similar to transport, the resting area or lairage may be a source of AMR for animals entering the slaughterhouse. Different batches of animals are housed within the same facilities and may come in contact with ARB present in the environment, as shown for pathogens (Rule et al., 2008). The burden of pathogens and ARB may relatively high in these holding pens (Walia et al., 2017), thus, there is a risk of introducing ARB into the slaughterhouse environment from contaminated skin and the intestines.

Slaughter line

Slaughtering activities may introduce or spread pathogens into the food chain (carcasses), and thus, they may serve as source of AMRs. As an example, cross-contamination of poultry at slaughter contributes to greater microbial diversity in retail chicken than in live birds (Davis et al., 2011; Althaus et al., 2017).

Manual or high throughput automated or semi-automated processes are potential sources of disseminating ARB. Defeathering and evisceration stages are considered the main sources of carcass contamination and release of microorganisms into the slaughter environment, mainly via spillage of intestinal contents and should be included in HACCP programmes as potential critical control points (CCP) to reduce the burden of ARB (Wu et al., 2009; Pacholewicz et al., 2015; Van Gompel et al., 2020).

Contamination of water and workers may also introduce/spread bacteria on the line (Gomes-Neves et al., 2012). The air flow and aerosols from slaughter facilities may also favour the spread of ARB and ARGs. A recent study has detected around 30% of air samples positive for *tet*(W) or *emr*B genes (Van Gompel et al., 2020). In contrast, other studies state that the transmission through air in slaughter facilities is negligible (Pearce et al., 2006; Okraszewska-Lasica et al., 2014). Burfoot et al. (2006) demonstrated that filtered air reduces carcass contamination, but also highlights that other factors (such as carcass handling or processing) provide better solutions to bacterial surface contamination. Occupational transmission of ARB and ARGs may occur at slaughterhouses (Mulders et al., 2010; van Cleef et al., 2010; Gilbert et al., 2012; Van Gompel et al., 2020). In addition, workers, through their hands or equipment, may act as sources of AMR (Van Gompel et al., 2020). Resistome studies have revealed not only frequent abundance of ARGs along the slaughter line but also some variability in these ARGs, emphasising the importance of good hygiene in carcass processing as stated at the beginning of this point (Campos Calero et al., 2018; Van Gompel et al., 2020).

Rendered poultry abattoir and hatchery waste, as well as low grade category 3 abattoir waste, can be used for manufacture of feed for pets (including raw meat pet food) and farmed fish. Occurrence of AMR in such products is a means of further dissemination of AMR beyond the food chain (Hofacre et al., 2001; Groat et al., 2016; Davies et al., 2019).

Process water and wastewater resulting from the slaughtering process can also be a source of contamination (Savin et al., 2020).

Processing plants

Carcasses are usually processed in cutting/processing plants where meat is processed to be delivered to retail establishments. In contrast to the abundance of information on the presence of food-borne pathogens in processing plants, which is well documented (Giovannacci et al., 2001; Argüello et al., 2013b), and of information on AMR on carcasses, studies performed on ARB and ARGs in processing plants, and particularly in the processing plant environment, are scarce. It can be assumed that meat introduced in the processing facilities acts as primary source of ARB and ARGs (Argüello et al., 2013a), which then can be indirectly spread during meat processing. Surfaces, equipment, workers and process water could be potential sources for ARB and ARGs if food hygiene procedures are not properly implemented. For instance, resistant *Listeria monocytogenes* strains were isolated from samples taken from equipment, washing sinks, disinfection tanks, surfaces and other environmental sources from meat and/or fish processing plants (Noll et al., 2018; Skowron et al., 2018; Rugna et al., 2021).

Much of the poultry meat that is used for further processing in many European countries is imported from third countries[33] where there are problems with the widespread emergence of AmpC resistance, e.g. in *Salmonella* Heidelberg. This presents a potential threat of introduction of this vertically transmitted organism into the EU poultry production environment via escape of organisms from processing waste or from infected humans, or pets consuming raw meat based foods (Souza et al., 2020; Dazio et al., 2021). A recent international *Salmonella* outbreak caused by powdered egg and linked to persistent contamination of a spray drying machine also illustrates the role of post-harvest contamination of the processing environment (Labska et al., 2021).

In dairy production, milk is normally collected for processing and only a small percentage is locally processed at the farm. Bacteria from the skin and intestinal microbiota or the environment (e.g. from bedding or other sources) may contaminate milk. The pasteurisation process is quite effective at eliminating pathogens and thereby also ARB. However, there are possibilities of transmission of ARB, also ARG, originating from the environment (soil and animals) to products consumed raw such as raw milk and artisanal cheeses. In fact, Alexa et al. (2020) performed a study showing that Gram-negative microorganisms of animal or soil origin dominated the microbiota of milk- and cheese-processing environments investigated.

3.1.2.3. Sector specific AMR sources and transmission routes

In the following sections, the sectors of poultry, cattle (dairy and beef/veal) and production are addressed individually. Schematic figures highlight the flow of AMR along the production chain and summarise sources and transmission routes for introduction of AMR into the production chain. Data on introduction of AMR (or of pathogens, if no information is available on AMR) is summarised in accompanying tables, per sector (poultry, Figure 8, Table 2; cattle, Figures 9–10, Table 3; and pigs, Figure 11, Table 4). In the Appendix E, the specific sectors are described in more detail.

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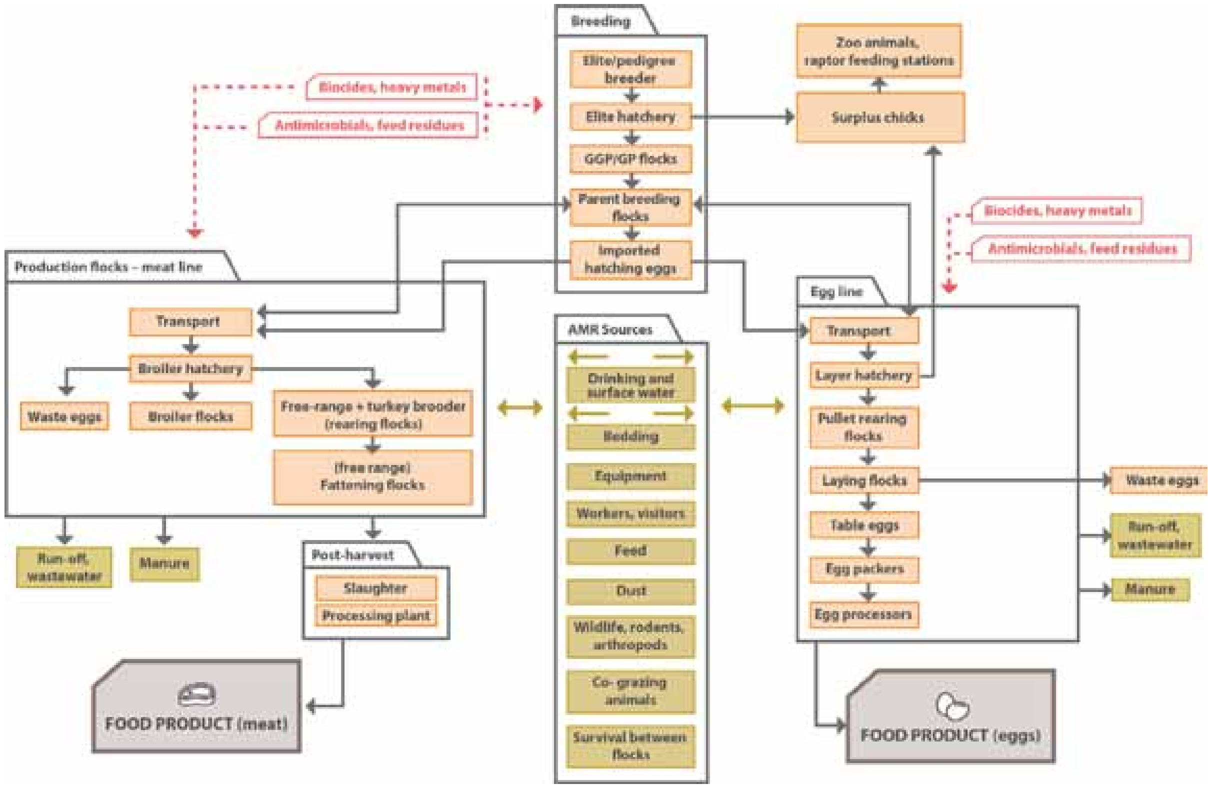
[33] https://ec.europa.eu/info/food-farming-fisheries/animals-and-animal-products/animal-products/poultry_en

3.1.2.3.1. *Poultry production sector*



Potential sources of AMR (resistant bacteria, both human pathogenic, zoonotic, commensal or environmentally associated and/or resistance genes) for the food production environment are shown as dark gold boxes, and transmission routes as dark gold arrows. AMR can either be introduced from these sources into the food chain, or AMR can also flow from the food production chain to these sources. Black arrows depict the flow of AMR along the food production chain (orange boxes). Red arrows depict the usage of antimicrobial agents (if applicable) and biocides and the presence and use of heavy metals in food production and its effect on AMR (selection of AMR). Workers and visitors signify people with access to the production environment, for either professional or other reasons. Wildlife includes all animals with access to the production chain (such as birds, larger mammals) but excludes pests typically associated with food production. Rodents include vertebrate pests such as rats and mice. Arthropods include non-vertebrate pests such as flies, other insects and beetles. Companion animals are limited to those animals having access to the production environment. Details for poultry post-harvest production are shown in Figure 7. Definitions of terms used are given in the glossary.

Figure 8: Environmental sources and transmission routes of AMR transfer - poultry production

Table 2: Stratification of environmental sources, transmission routes and risk factors of AMR – poultry production sector

Farm	Meat production	Egg production	Supporting references	Comments and uncertainties
Feed	+	+	Österberg et al. (2006), Sapkota et al. (2007), Ge et al. (2013), Rossato et al. (2019)	No studies have followed acquisition of ARB by animals from feed, apart from *Salmonella.*
Bedding	?/+	?/+	Yang et al. (2006)	No data on the fate of AMR organisms in unused poultry litter but the same bedding materials that are used for poultry that were assessed in a dairy farm context harboured significant bacterial counts.
Drinking water	?/+	?/+	Khan et al. (2016)	No specific data on ARB in poultry drinking water – but will be likely to occur as bacterial contamination and biofilm is common in water lines and many poultry farm environmental organisms are AMR. Water sourced from bore holes is more likely to be contaminated as a result of environmental contamination of surface water.
Surface water	?/-	?/-	Maes et al. (2019), Wu et al. (2020)	Rarely direct access for poultry.
Dust/air	?/+	?/+	Gao et al. (2017), Luiken et al. (2020), Wychodnik et al. (2020)	Air is likely to reflect contaminated dust - only circumstantial evidence of dust as a source. As above, plus antimicrobials also disseminated in dust from poultry flocks.
Wildlife, rodents, arthropods	?/+	?/+	Dolejska (2020)	Mainly acting as vectors – role as primary reservoir can't be quantified.
Co-grazing animals	?/+	?/+		No specific info on co-grazing risk for poultry - but ARB exposure will be as for horses, sheep and camelids that are co-grazed with free range poultry.
Companion animals	?/+	?/+	Davies et al. (2019)	Increased risk if fed raw meat pet foods, but no published evidence as a source for poultry.
Equipment	?/+	?/+	Dame-Korevaar et al. (2019)	Publication confirms variety of secondary introduction routes, including from contaminated equipment, and inability to quantify their contribution to AMR.
Poor biosecurity on poultry farms allowing entry via various pathways	++	+	Davies and Wales (2019)	Based on *Salmonella* and *Campylobacter*, plus limited AMR risk factor studies.
Breeding (importation of breeding stock)	++	+	Dierikx et al. (2013), Börjesson et al. (2016)	Some good evidence of carriage of ARB by imported chicks exists, particularly for *Salmonella* and ESBL producing *E. coli*
Survival between flocks (ineffective disinfection)	++	+	Aksoy et al. (2020)	Based on *Salmonella* and *Campylobacter*, plus limited risk factor analysis and field studies.

Farm	Meat production	Egg production	Supporting references	Comments and uncertainties
Antimicrobials in hatcheries	++	+	Wales and Davies (2020)	Numerous studies report a temporal correlation between introduction and cessation of routine AMU in breeding flocks or hatcheries and occurrence or decline of AMR in Salmonella or *E. coli.*
Antimicrobial usage in commercial flocks	++	+	Pesciaroli et al. (2020)	AMU in poultry flocks is rapidly followed by selection of specific AMR and MDR in *E. coli.*
Biocides (in hatcheries)	?/+	?	Davies and Wales (2019)	Selection of AMR by biocides has not been proven in field studies, only in the laboratory and unpublished observations from the field.
Post harvest/handling	**Meat production**	**Egg production**	**Supporting references**	**Comments and uncertainties**
Equipment/air	+	+	Davies and Breslin (2003), Buess et al. (2019)	Cross-contamination of poultry meat products during the working day, and sometimes over longer periods, as a result of contaminated equipment and air is a common occurrence, with the extent of contamination being influenced by slaughter and processing practices. Egg shell contamination can be transferred from egg packing equipment.
Abattoir/processing waste handling	?/+	?/+	Savin et al. (2020)	Poultry processing waste is highly contaminated by AMR organisms, but there is no data to indicate whether this could be a source for poultry, e.g. via aerosol contamination or transport crates, which have been found to be contaminated by MDR *Salmonella* on arrival at poultry farms.

Importance of the single sources is given from – (not important) to + (represents a source/factor of AMR, evidence for introduction existing for other bacteria), ++ (important source/factor of AMR). ?: specific information is missing. ?/– No scientific evidence but presumably no source/factor, ?/+ No scientific evidence but presumably source/factor as AMR is present in the source and introduction to the farm is possible. Importance is assigned based on the presence of supporting references and expert assessment, as scientific evidence on the relative attribution of AMR to the specific environmental sources is generally lacking. Dark gold: environmental sources, red: other selective pressures/risk factors.

Uncertainties

The consideration of the importance of the sources in Table 2 above is based largely on expert opinion and extrapolation from evidence of transmission of food-borne pathogens such as *Salmonella* or *Campylobacter.* Although there is a small amount of information on the occurrence of AMR commensal organisms in the poultry breeding and production chain, a causal relationship between the finding of the same organism in the poultry environment and its occurrence in birds has to be assumed. This is because there are no prospective studies that demonstrate colonisation was actually caused by exposure to specific contaminated environmental elements, rather than contamination of the environment with pathogens originating from the birds or another unrecognised source. There are also no robust intervention studies to conclusively prove interruption of ongoing patterns of infection after eliminating ARB from the production environment, so all current evidence is based solely on observations of potential correlations, even though this evidence may be true in many cases. It is therefore impossible to demonstrate the direction of contamination with certainty, or to exclude other undetected sources, although several observational or epidemiological studies, supported by molecular biology, do provide strong circumstantial evidence of a likely environmental association. Most of post-harvest studies are focused on the study of carcass contamination. More research is needed to evaluate the impact of the post-harvest environment in meat contamination of ARB. For more information see Appendix D, Table D.1.

3.1.2.3.2. *Cattle (dairy and beef/veal) production sector*

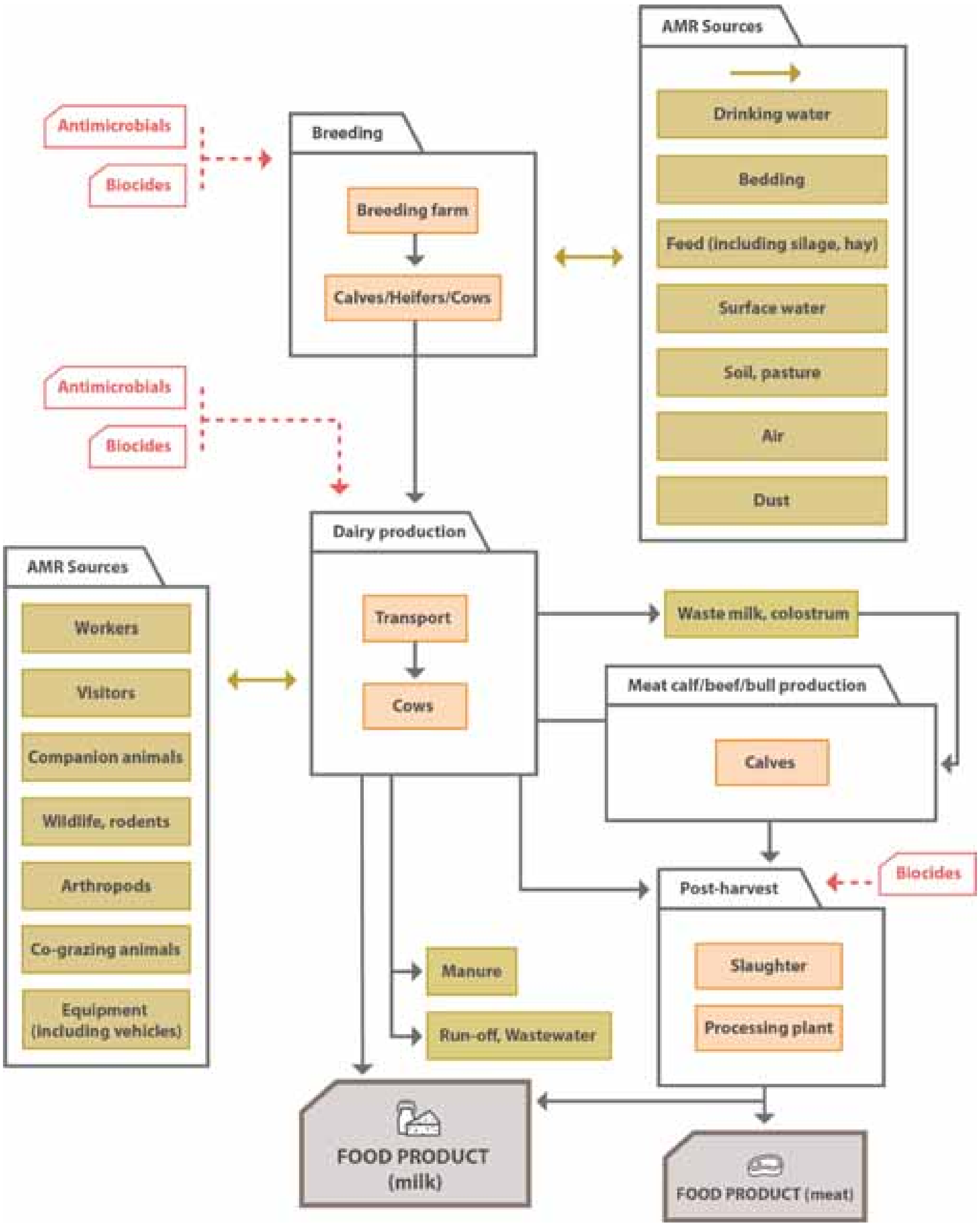


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Figure 9: Detailed environmental sources and transmission routes of AMR - dairy production

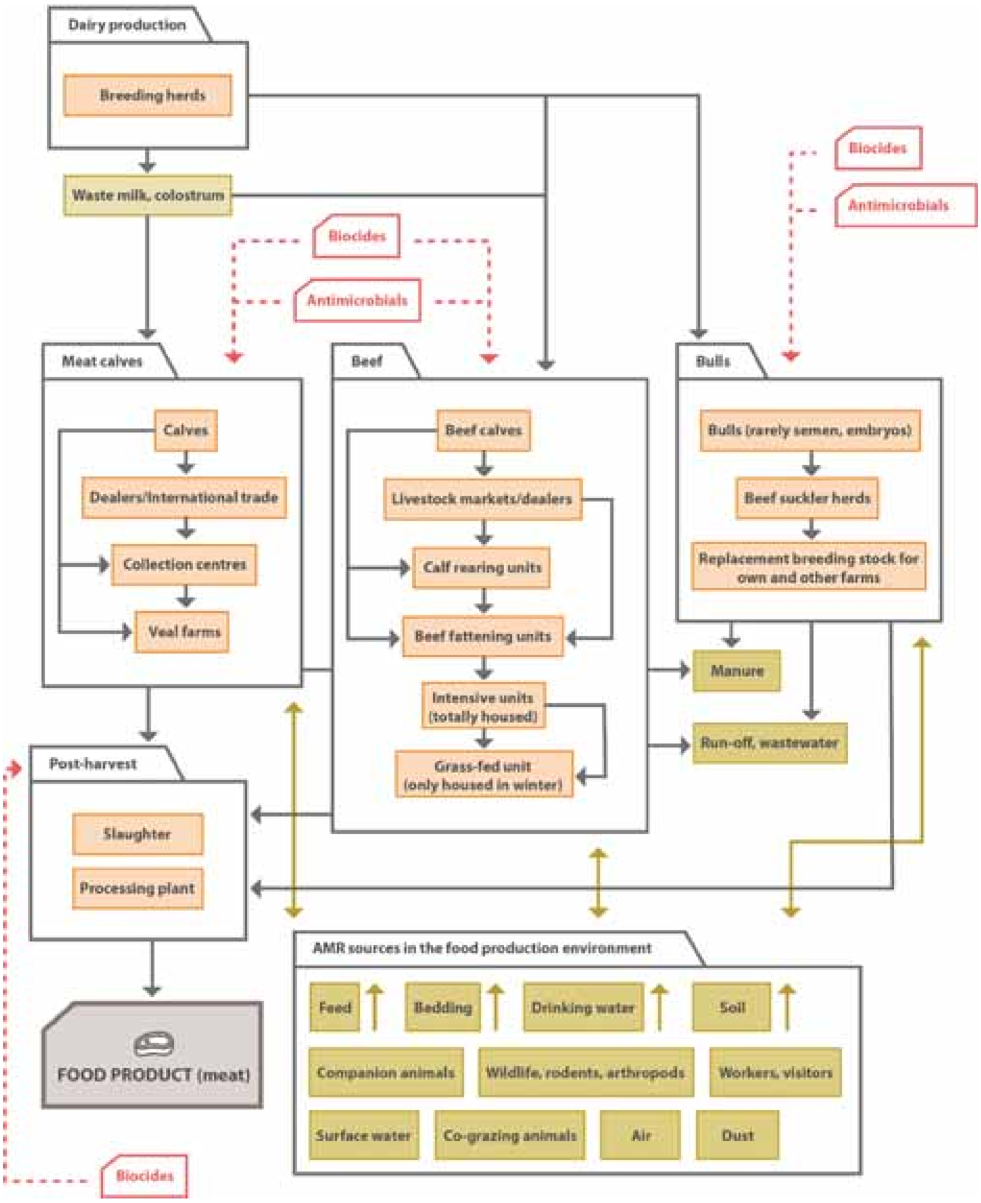

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Figure 10: Detailed environmental sources and transmission routes of AMR – cattle (beef/veal) meat production

Table 3: Stratification of environmental sources, transmission routes and risk factors of AMR – cattle production[(a)]

AMR sources	**Grazing animals**				**Closed farm**				**Supporting references**	**Comments and uncertainties**
Primary production	**Cows/ heifers**	**Calves**	**Beef cattle**	**Milk**	**Cows/ heifers**	**Calves**	**Beef cattle**	**Milk**		
Human	++	+	?/+	++	++	+	?/+	+	Schnitt and Tenhagen (2019)	Human contamination is hard to trace, however, some evidence of human introduction of MRSA, especially in the instances where CA- and HA- MRSA were isolated from cattle.
Equipment (milking) and procedures	?/+	–	–	?/+	?/+	–	–	?/+		Equipment could be contaminated by environmental bacteria and from the milk contaminated with ARB during the milking process.
Waste milk	–	++	–	–	–	++	–	–	EFSA BIOHAZ Panel (2017), Tetens et al. (2019), Springer et al. (2019)	
Feed	?/	?/	?/+	–	?/	?/	?/+	–		Feed references were not found but likely source.
Silage/other greens	?/+	–	?/+	–	?/+	–	?/+	–		Lacking information but likely source.
Pasture contamination	?/+	?/+	?/+	?/+		–	?/+	–	Markland et al. (2019)	Pasture soil from farms with high levels of cephalosporin resistance in beef calves contained a large proportion of Proteobacteria as well as high CFU counts of cephalosporin-resistant bacteria, even though antimicrobial use was kept low.
Water	?/+	?/+	?/+	?/+	?/+	?/+	?/+	?/+	Duse et al. (2016)	
Bedding/soil	+	+	?/+	+	++	++	?/+	++	Subbiah et al. (2012), Astorga et al. (2019)	
Air/dust	?/+	?/+	?/+	?/+	?/++	?/++	?/+	?/+	Schmid et al. (2013), Tenhagen et al. (2014), Navajas-Benito et al. (2017)	

AMR sources	Grazing animals				Closed farm				Supporting references	Comments and uncertainties
Primary production	**Cows/ heifers**	**Calves**	**Beef cattle**	**Milk**	**Cows/ heifers**	**Calves**	**Beef cattle**	**Milk**		
Wildlife	?/+	?/+	?/+	?/+	?/+	?/+	?/+	?/+	Arnold et al. (2016), Tormoehlen et al. (2019)	Limitations on species studied and seasonal variation.
Co-grazing animals/mixed farms	++	+	?/+	+	?/–	?/–	?/–	?/–	Hansen et al. (2019)	
Proximity other animal farms	++	++	?	+	?/+	?	?	?	Locatelli et al. (2017)	
Companion animals	?/+	?/+	?/+	?/+	?/–	?	?	?		Limited exposure unless given access to farm premises.
Antimicrobials	++	++	++	++	++	++	++	++	Jayarao et al. (2019)	Quite variable.
Acquisition of animals	++	++	++	++	+	++	++	–	Dantas Palmeira and Ferreira (2020)	
Post harvest: Handling/processing							**Meat**			
Water							?/+			
Humans							?/+			
Wildlife							?/–			
Machinery, materials equipment							?/+		Papadopoulos et al. (2019)	
Air/dust								?/+	Okraszewska-Lasica et al. (2014)	

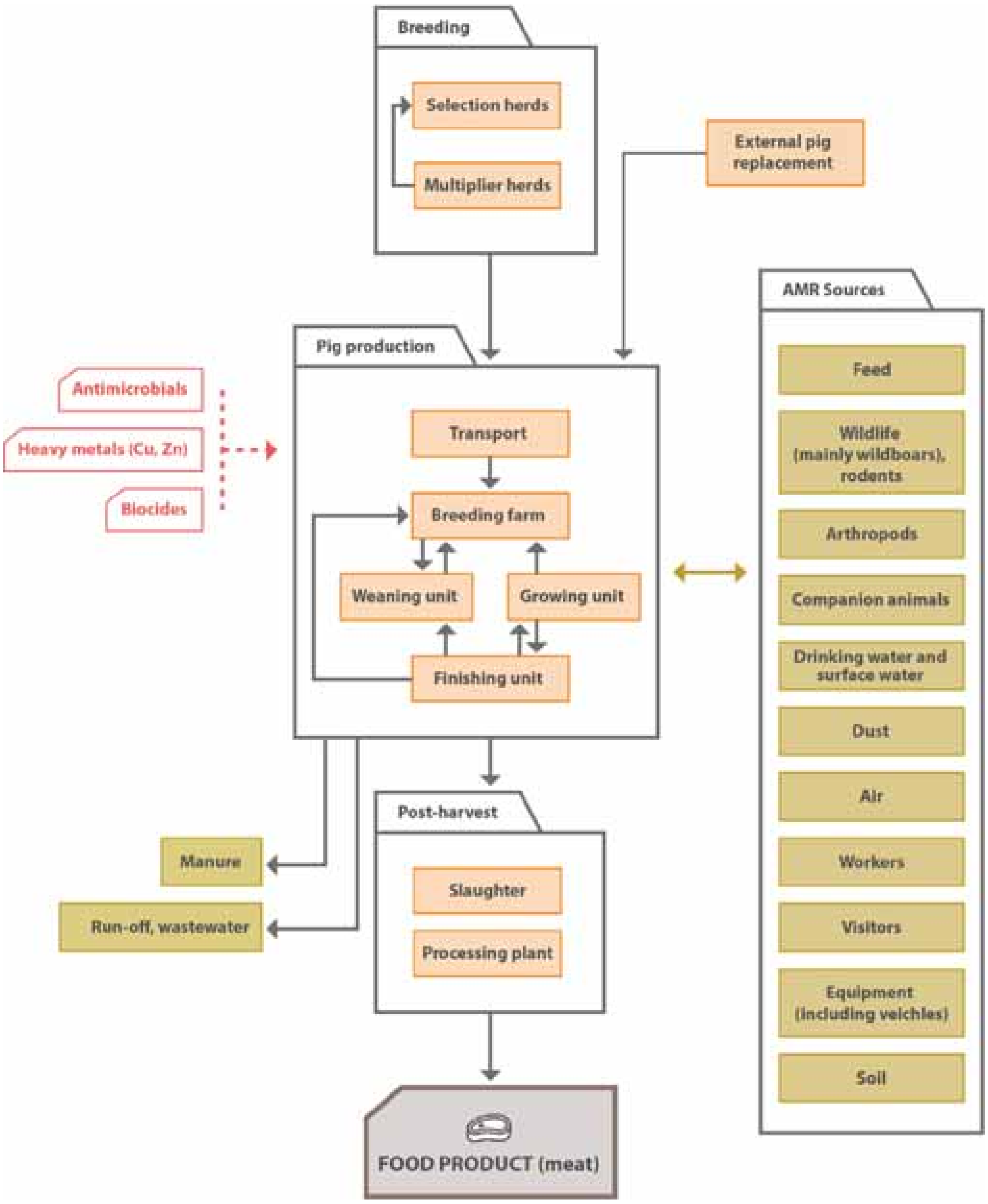
Importance of the single sources is given from – (not important) to + (represents a source/factor of AMR, evidence for introduction existing for other bacteria), ++ (important source/factor of AMR). ?: specific information is missing. ?/– No scientific evidence but presumably no source/factor, ?/+ No scientific evidence but presumably source/factor as AMR is present in the source and introduction to the farm is possible. Importance is assigned based on the presence of supporting references and expert assessment, as scientific evidence on the relative attribution of AMR to the specific environmental sources is generally lacking. Dark gold: environmental sources, red: other selective pressures/risk factors.

(a): A closed farm is breeding its own replacements and does not bring in replacement animals from outside. Relevance of the sources is given for introduction into the dairy (Cows/heifers) and meat production sector (calves, beef cattle). Also, sources are specified that are of relevance for contamination of milk.

Uncertainties

While there is (limited) information on the occurrence of AMR organisms in cattle/milk production as related to environmental sources, a causal relationship between the finding of the same organism in the production environment and its occurrence in bovine animals has often not been substantiated. This is because there are no prospective studies that demonstrate colonisation was actually caused by exposure to specific contaminated environmental elements, rather than contamination of the environment with pathogens originating from the animals or another unrecognised source. There are also no robust intervention studies that conclusively demonstrate a reduction of ARB carriage after eliminating specific sources of ARB from the production environment, so all current evidence is based solely on observations of potential correlations. Furthermore, there is insufficient quantitative information on transmission of ARB and ARGs from one specific source, thus a ranking of the importance of the sources is based on expert evaluation. Most post-harvest studies are focused on the study of carcass contamination. More research is needed to evaluate the impact of the post-harvest environment for meat contamination. For more information see Appendix D, Table D.1.

3.1.2.3.3. Pig production sector



Potential sources of AMR (resistant bacteria, both human pathogenic, zoonotic, commensal or environmentally associated and/or resistance genes) for the food production environment are shown as dark gold boxes, and transmission routes as dark gold arrows. AMR can either be introduced from these sources into the food chain, or AMR can also flow from the food production chain to these sources. Black arrows depict the flow of AMR along the food production chain (orange boxes). Subcategories of the production chains are shown as folders. Red arrows depict the usage of antimicrobial agents and biocides and the presence or use of heavy metals in food production and its effect on AMR (selection of AMR). Workers and visitors signify people with access to the production environment, for either professional or other reasons. Wildlife includes all animals with access to the production chain (such as birds, larger mammals) but excludes pests typically associated with food production. Rodents include vertebrate pests such as rats and mice. Arthropods include non-vertebrate pests such as flies, other insects and beetles. Companion animals are limited to those animals having access to the production environment. Surface water, pasture and soil are only relevant if animals have access to it, e.g. during free ranging. Run-off signifies faecal contamination of adjacent surface waters when animal faecal matter is washed off the production environment with rain. Wastewater represents e.g. water used to clean the production or slaughterhouse environment that is then discharged into the canalisation or treated on-site before release to surface water. Details for pig post-harvest production are shown in Figure 7. Definitions of terms used are given in the glossary.

Figure 11: Detailed environmental sources and transmission routes of AMR – pig production

Table 4: Stratification of environmental sources, transmission routes and risk factors of AMR - pig production

Farm	**Indoor farms**	**Outdoor/ extensive farms**			**Supporting references**	**Comments and uncertainties**
Feed (ingredients and/or compound feed)	+	+			Molla et al. (2010), Novais et al. (2013), Burns et al. (2015), Mourão et al. (2015)	Minimal research on AMR apart from specific pathogens such as *Salmonella* and *Enterococcus.*
Drinking water	?	?				Lack of data to support or dismiss water as AMR environmental source.
Bedding	?	?				Lack of data to support or dismiss bedding as environmental source.
Air/Dust	?/+	?/+			Gibbs et al. (2004), Novais et al. (2013), Braga et al. (2013), Luiken et al. (2020)	
Wild animals	?/+	?/++			Allen et al. (2011), Andrés et al. (2013), Dias et al. (2015), Bonardi et al. (2019)	Different studies support the carriage of ARB by wild animals. Wild boars are of particular relevance for pigs. Outdoor farming may favour the contact between farmed and wild animals, increasing the risk.
Visitors/farmers	?	?				Lack of specific studies.
Outdoor soil		?/+			Novais et al. (2013)	The study collects samples from soil where *Enterococcus* spp., is isolated.
Biosecurity measures other than cleaning and disinfection	+	?/+				Lack of specific studies on the impact of biosecurity in the rise and spread of AMR in the farm environment.
Cleaning and disinfection	+	?			Martelli et al. (2017)	Lack of studies which evaluate the impact of C&D on the presence of AMR in the environment, but can impact MDR *Salmonella*. Hard to implement in outdoor extensive farms.
Selection herds	?	?			EFSA (2008c), Argüello et al. (2013c)	Different studies highlight the presence of ARB in selection herds. No data about the risk of transmission in the production pyramid.

Farm	Indoor farms	Outdoor/ extensive farms			Supporting references	Comments and uncertainties
Mother to progeny	?/+	?/+			Lynch et al. (2018)	
Replacement	++	++			Sieber et al. (2018)	
Antimicrobials	++	++			EMA and EFSA (2017), Munk et al. (2018)	Clearly the main influencing factor in AMR development, also contributes to presence of ARB in the environment.
Heavy metals	?/+	?/+			Hölzel et al. (2012), EFSA FEEDAP Panel (2016)	Although heavy metals used in feed could co-select for AMR, more evidence is needed under field conditions. For compounds of trace elements (Cu, Zn) used as feed additives, the EFSA FEEDAP Panel concluded that 'a co-selection in the gut bacteria for resistance to copper and resistance to erythromycin cannot be excluded'.
Selection by biocides	?	?			FAO/WHO (2018)	Although biocides could co-select for AMR, more evidence is needed under field conditions.
Post-harvest	**Transport**	**Lairage**	**Slaughterhouse**	**Processing plant**		
Process water	?	?	?	?		Lack of data to support or dismiss process water as an AMR source during post-harvest.
Workers/handling activities			?/++	+	Gomes-Neves et al. (2012), Melero et al. (2012), Van Gompel et al. (2020)	More research is needed to evaluate the role of these activities although they could be source of ARB by the few literature available.
Air			?/+	+	Pearce et al. (2006), Okraszewska-Lasica et al. (2014), Van Gompel et al. (2020)	
Surfaces			?	+	Sala et al. (2016)	

Importance of the single sources is given from – (not important) to + (represents a source/factor of AMR, evidence for introduction existing for other bacteria), ++ (important source/factor of AMR). ?: specific information is missing. ?/– No scientific evidence but presumably no source/factor, ?/+ No scientific evidence but presumably source/factor as AMR is present in the source and introduction to the farm is possible. Importance is assigned based on the presence of supporting references and expert assessment, as scientific evidence on the relative attribution of AMR to the specific environmental sources is generally lacking. Dark gold: environmental sources, red: other selective pressures/risk factors.

Uncertainties

References are quite limited and do not sustain stratification or quantitative estimates on the table. The direction of the relationship between the finding of the same organism in the pig environment and its occurrence in pigs has to be assumed. This is because there are no prospective studies that demonstrate pig colonisation was actually caused by exposure to specific contaminated environmental elements, rather than contamination of the environment with pathogens originating from the pigs. Neither are there intervention studies to show interruption of ongoing patterns of infection after eliminating ARB from the production environment. Therefore, stratification of the role of different sources and transmission pathways is difficult. Most of post-harvest studies are focused on the study of carcass contamination. More research is needed to evaluate the impact of post-harvest environment in meat contamination. For more information see Appendix D, Table D.1.

3.1.3. Aquaculture production sector

The EU total production of aquaculture products was estimated to be 1.1 million tonnes live weight in 2018 (Eurostat),[30,34] of which a few MSs accounted for the majority of the production (Spain, France, Italy and Greece). Norway (non-EU MS) is the leading aquaculture producer in Europe with a total production of 1.4 million tonnes. European aquaculture production covers a wide range of production systems and species, including coastal and freshwater finfish production (mostly Atlantic salmon), small scale land based saltwater production systems, coastal and estuarine bivalve mollusc (e.g. oysters and mussels) production and small scale crustacean production.

Freshwater species are cultivated either extensively in ponds or intensively in tanks (e.g. rainbow trout). There are two different techniques of tank production for intensive farming, continuous flow systems and recirculation aquaculture systems (RAS) where water is nearly fully recycled and remains in the tanks. RAS are cost-intensive due to high energy usage but offer regulation of breeding conditions and can also be used for cultivation of marine species. Marine species are reared either in shore-based tanks in a controlled environment with recirculating seawater or in cages in sheltered zones near shore (e.g. Atlantic salmon). For shellfish cultivation, different techniques such as ropes, wooden posts, tables or bottom-farming are used. The diversity of production systems, in different environments and subject to a wide range of pollution sources, means that aquaculture production has the potential to be contaminated by significant bacterial and chemical pollution. Open systems, such as continuous flow systems or cages are a particular environmental concern when discharges containing antimicrobial residues or feed/faeces are released into the environment (Muziasari et al., 2017).

There has been significant discussion about antimicrobial use in finfish aquaculture in Europe, with claims that due to low usage the risk of development of AMR is negligible (Lillehaug et al., 2018). However, as usage concentrations in aquaculture are several orders of magnitude higher than concentrations of environmental residues derived from livestock and human excretion, the relatively small amounts used (212 kg in Norway in 2016) may have increased significance in terms of driving selection for AMR in aquatic environments including aquaculture production systems (Bailey and Eggereide, 2020).

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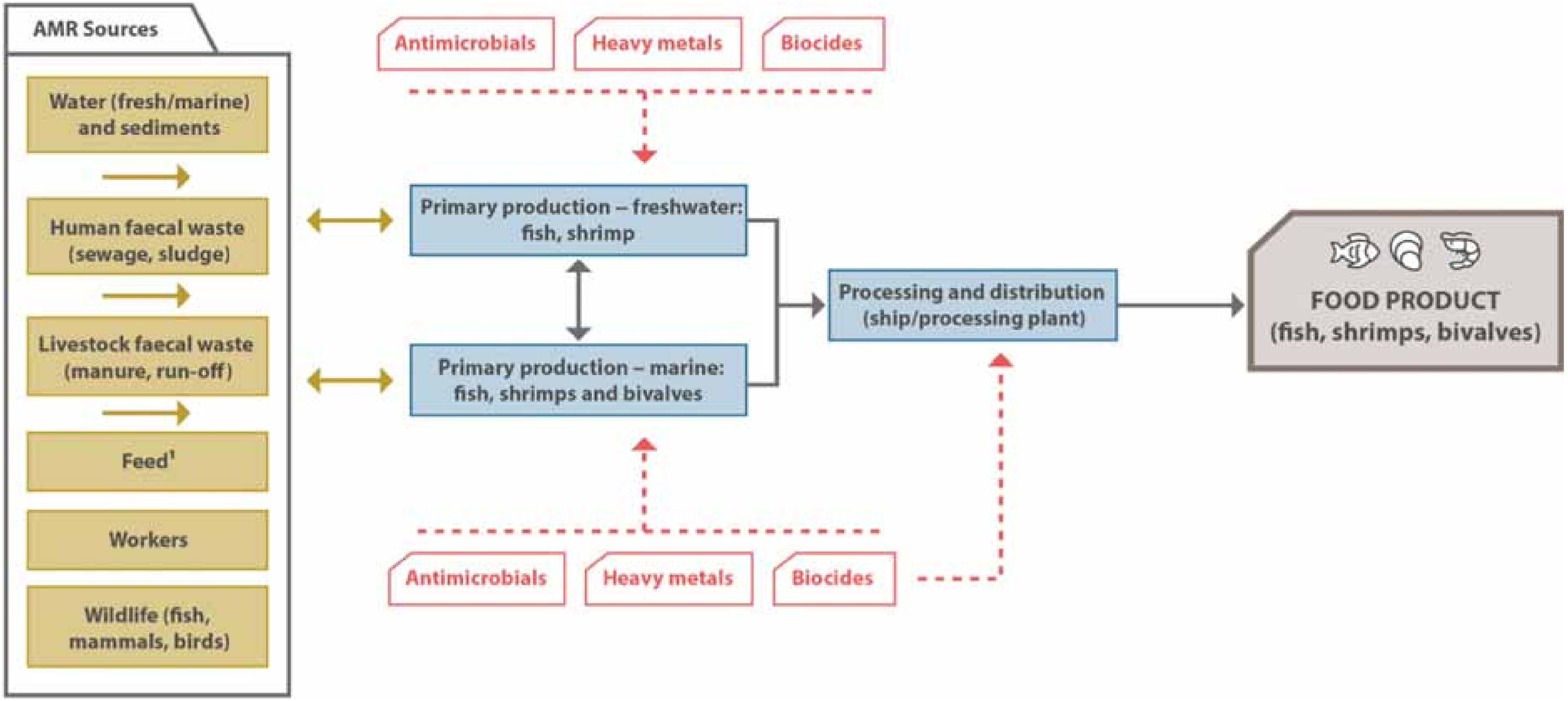
[34] https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Fishery_statistics (last accessed, February 2020).



(1): Only for fish and shrimps, not for bivalves.
Potential sources of AMR (resistant bacteria, both human pathogenic, zoonotic, commensal or environmentally associated and/or resistance genes) for the food production environment are shown as dark gold boxes and transmission routes as dark gold arrows. AMR can either be introduced from these sources into the food chain, or AMR can also flow from the food production chain to these sources. Black arrows depict the flow of AMR along the food production chain (blue boxes). Subcategories of the production chains are shown as folders. Red arrows depict the usage of antimicrobial agents and biocides and the presence of heavy metals in food production systems and its effect on AMR (selection of AMR). Workers and visitors signify people with access to the production environment, for either professional or other reasons. Wildlife includes all animals with access to the production chain (such as birds, larger mammals) but excludes pests typically associated with food production. Definitions of terms used are given in the glossary.

Figure 12: Detailed environmental sources and transmission routes of AMR – aquaculture production

3.1.3.1. Preharvest AMR sources and transmission routes

Preharvest environmental sources and transmission of AMR (see Figure 12) may differ for different types of aquaculture. In general, production systems rely on a high-quality aquatic environment (water and sediment) and unlike most livestock production the growth medium (i.e. water) comes from outside the farm and may be subject to a wide range of pollution including municipal wastewater, sewage (untreated and treated), run-off and sub-surface flow from urban and agricultural land. These sources can contain a wide range of bacterial and chemical pollutants, including ARB and ARG from human faeces and from animals via wash water, abattoir wastewater, antimicrobials, heavy metals and biocides, plus a wide range of other pharmaceutical and plant protection product residues (Müller et al., 2020; Radisic et al., 2020).

Bivalve molluscs, including mussels and oysters, filter large volumes of water and can concentrate particles and pathogens and are therefore uniquely vulnerable to bacterial contamination of river and coastal water, including with ARB, even though antimicrobials are not routinely used in their production. E.g. a study of *Clostridioides difficile* in wild and farmed shellfish in Italy found antimicrobial-resistant *C. difficile,* including toxigenic strains causing human disease and strains associated with cattle and pigs (Agnoletti et al., 2019).

Generally, water is a major route for disseminating ARB and was reported as a source of AMR in rainbow trout cultivation in Portugal (Novais et al., 2018). Fish farms have also been implicated in increased levels of AMR in European river systems, with a study in Brittany showing enrichment of oxolinic acid and oxytetracycline-resistant *Aeromonas* spp. in river water immediately downstream of fish farms (Gordon et al., 2007). Most studies only focus on a limited number of bacterial taxa or resistance phenotypes or ARGs. However, an experimental study of AMR associated with fish production in Italy demonstrated that in groundwater-fed fish breeding tanks, for three different salmonids, water contained a wide range of ARGs including those conferring resistance to several antimicrobials (Colombo et al., 2016). Unfortunately, the latter study did not analyse the influent ground water or the feed; however, it does demonstrate diverse AMR associated with fish microbiomes in the absence of antimicrobial usage. Wildlife, domestic animals, human populations and natural environments are closely interconnected, and all play a role in AMR dynamics (Vittecoq et al., 2016). There is a large amount of literature detailing AMR carriage by wildlife, including wild birds such as seagulls which often carry clinically important AMR, presumably due to their diverse feeding habits on landfill, agricultural and wastewater impacted environments (Radhouani et al., 2011). However, studies of AMR in aquaculture where wildlife are attributed as sources are very rare.

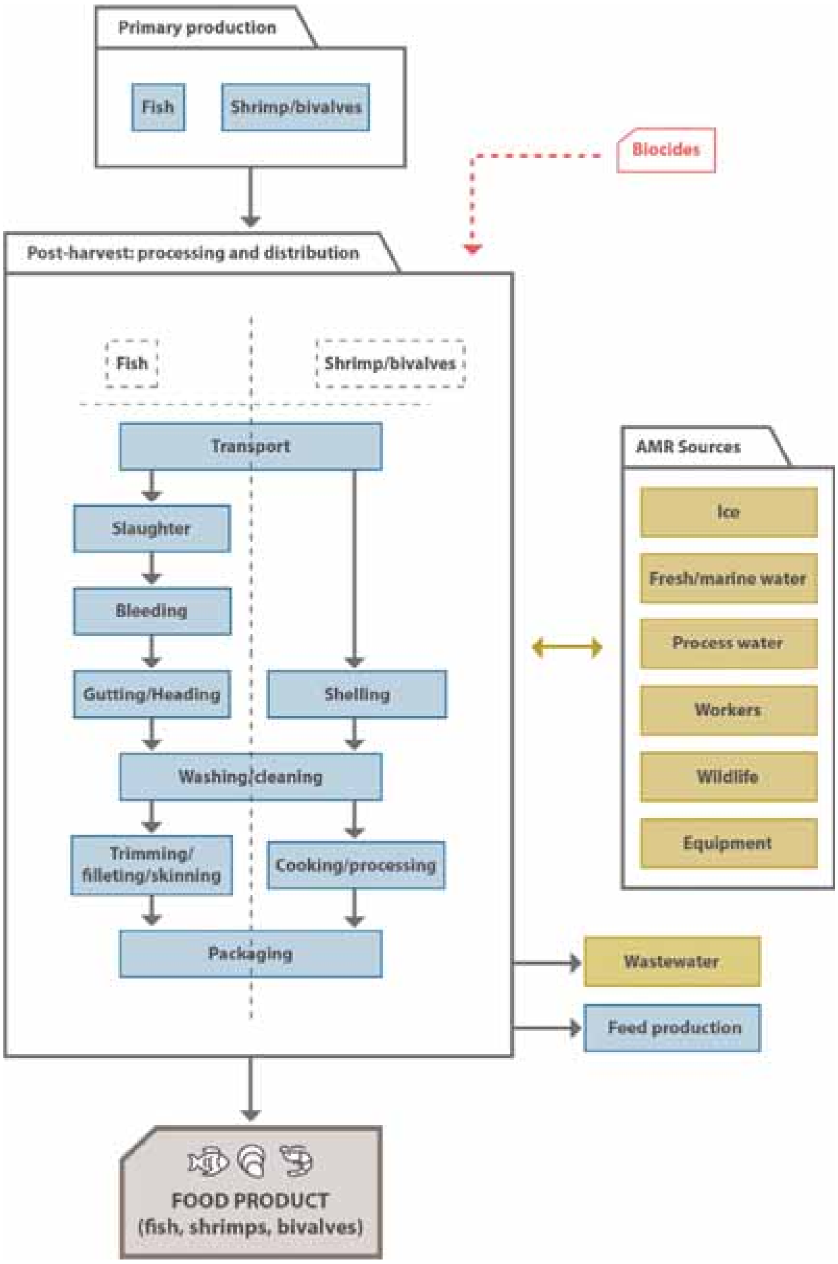
Additionally, fish feed may serve as source of contamination, e.g. *Enterococcus* strains (particularly multidrug resistant *E. faecium*) were isolated from feed in Portugal (Novais et al., 2018) and *Salmonella* can colonise fish feed production facilities (Møretrø et al., 2003) or be recycled via fish meal (Lunestad et al., 2007).

Fish handlers can become carriers of pathogens when they are infected e.g. with salmonellosis. As *Salmonella* are excreted in faeces, poor personal hygiene of workers can lead to contamination of fish during preparation or processing steps (see below). Generally, contamination of fish products can emerge during all processing stages (transportation, contact with contaminated water or tools, etc., see below) (Fernandes et al., 2018).

3.1.3.2. Post-harvest sources and transmission routes

Data on post-harvesting sources of bacterial AMR in the EU are scarce, and focused on *L. monocytogenes* (Skowron et al., 2018). Contamination during post-processing for fish will most likely occur during slaughter, gutting, trimming and filleting. Based on expert judgement contamination is likely to originate from the contaminated fish (e.g. through contaminated fresh or marine water), the workers, unclean equipment, unclean water resulting from the processing or ice used for transportation and wildlife. This is especially important for those species of aquaculture such as bivalves and shrimps, which enter the food chain without extended food processing and where their gut content may be part of the food. It is likely that this gut content will provide a transfer route for ARB, but so far documented data on this possible transfer is largely lacking, particularly in the EU.

Evidence for the above routes of introduction of AMR into aquaculture is collated in Table 5. This includes an expert assessment of the relative importance of the different routes based on the quality of the evidence.



Potential sources of AMR (resistant bacteria, both human pathogenic, zoonotic, commensal or environmentally associated and/or resistance genes) for the food production environment are shown as dark gold boxes and transmission routes as dark gold arrows. AMR can either be introduced from these sources into the food chain, or AMR can also flow from the food production chain to these sources. Black arrows depict the flow of AMR along the food production chain (blue boxes). Subcategories of the production chains are shown as folders. Red arrows depict the usage of antimicrobial agents and biocides and the presence of heavy metals in food production systems and its effect on AMR (selection of AMR). Workers and visitors signify people with access to the production environment, for either professional or other reasons. Wildlife includes all animals with access to the production chain (such as birds, larger mammals) but excludes pests typically associated with food production. Definitions of terms used are given in the glossary.

Figure 13: Detailed environmental sources and transmission routes of AMR – aquaculture processing

Table 5: Stratification of environmental sources, transmission routes and risk factors of AMR - aquaculture production sector

AMR Sources/risk factors aquaculture	Fresh water		Marine water			Supporting references	Comments and uncertainties
	Fish	**Shrimps**	**Fish**	**Shrimps**	**Bivalves**		
Primary production							
Water (fresh marine) and sediments						Lupo et al. (2012), Berendonk et al. (2015), Leonard et al. (2015), Bürgmann et al. (2018), Novais et al. (2018), Antunes et al. (2018), Zago et al. (2020)	Water, and associated sediment is a major route for disseminating ARB and ARGs that can originate from sources such as human faecal waste and/or animal faecal waste. Often the pollution source is not investigated or known. Water and sediment also contain indigenous bacteria, including fish pathogens with intrinsic AMR that may pose a threat to human health.
• Human faecal waste (sewage and sludge).	++	?/+	+	?/+	++		
• Livestock faecal waste (manure and run-off)	+	?	+	?/+	++		
Feed	+	+	+	+	?/–	Cabello (2006), Muziasari et al. (2017), Novais et al. (2018)	AMR has been reported as a contaminant of fish meal which is traded internationally and makes up a major component of feed in aquaculture systems.
Workers	?/+	?/+	?/+	?/+	?/+	Fernandes et al. (2018)	The mentioned literature relates to *Salmonella* and documents a transfer of AMR pathogens from fish products to workers not the other way around. That workers will have an impact on the aquaculture itself seems rather unlikely, especially in comparison to other sources.
Wildlife (fish, mammals, birds)	?/+	?/+	+	?/+	?/+	Radhouani et al. (2011), Di Cesare et al. (2013)	
Antimicrobials	+	?/+	+	?/+	?/–	Samuelsen et al. (1991), Rico et al. (2019)	Contamination originates mostly from water (except for the antimicrobials in fish feed). Direct publications on the shrimps in Europe are not available.
Heavy metals	+	?/+	+	?/+	?/+	Costello et al. (2001), Tornero and Hanke (2016), Bannister et al. (2019)	Heavy metals are used as antifouling agents on marine aquaculture infrastructure and historically also as molluscicides.
Biocides	?/+	?/+	?/+	?/+	?/+	Costello et al. (2001), Tornero and Hanke (2016)	Biocides are used as disinfectants in many aquaculture systems.

AMR Sources/risk factors aquaculture	Fresh water		Marine water			Supporting references	Comments and uncertainties
	Fish	Shrimps	Fish	Shrimps	Bivalves		
Post-harvest (transport and processing)							
🟨 Ice	?/+	?/+	?/+	?/+	?/–	Fernandes et al. (2018), Vaiyapuri et al. (2019)	The citations do not focus on European aquaculture, here we did not find any information. Most literature only details contamination with pathogenic bacteria, which is a well-known phenomenon.
🟨 Fresh/marine water	?/+	?/+	?/+	?/+	?/+	Fernandes et al. (2018), Vaiyapuri et al. (2019)	The citations do not focus on European aquaculture, here we did not find any information. Most literature only details contamination with pathogenic bacteria, which is a well-known phenomenon.
🟨 Process water	?/+	?/+	?/+	?/+	?/+		Lack of data to support or dismiss process water as an AMR source during post-harvest.
🟨 Workers	?/+	?/+	?/+	?/+	?/+		Lack of data to support or dismiss workers as an AMR source during post-harvest.
🟨 Wildlife	?/+	?/+	?/+	?/+	?/+		Lack of data to support or dismiss wildlife as an AMR source during post-harvest.
🟨 Equipment	?/+	?/+	?/+	?/+	?/+		Lack of data to support or dismiss equipment as an AMR source during post-harvest.
🟥 Biocides	?/+	?/+	?/+	?/+	?/–		Lack of data to support or dismiss biocides as an AMR source during post-harvest.

Importance of the single sources is given from – (not important) to + (represents a source/factor of AMR), ++ (important source/factor of AMR). ?: specific information is missing. ?/– No scientific evidence but presumably no source/factor, ?/+ No scientific evidence but presumably source/factor. Importance is assigned based on the presence of supporting references and expert assessment, as scientific evidence on the relative attribution of AMR to the specific environmental sources is generally lacking. Dark gold: environmental sources, red: other selective pressures/risk factors.

Uncertainties:

The table contains mainly two uncertainties: concerning aquaculture in Europe the literature is very scarce therefore we drew some conclusions from data on international aquaculture. Additionally, a further uncertainty concerns possible contamination routes via surface water. Here studies exist that focus on the quality of the surface water as affected by contaminants, but publications connecting the quality of surface water linked to its impact on the presence of AMR in aquaculture are lacking. For more information see Appendix D, Table D.1.

3.1.4. Concluding remarks regarding sources and transmission routes for antimicrobial-resistant bacteria and resistance genes in food-producing environments

- For all sectors, there is limited data on the introduction of ARB and ARG from most environmental sources into the production chain. Often, knowledge is limited to the presence of ARB and ARG in the sources. In some studies, there is evidence for the introduction of pathogens - but without information on antimicrobial resistance profiles or ARGs - from the sources into the production chain. In other cases, similar ARB have been found within the production environment and in the surrounding environment, but conclusive evidence of the origin and direction of dissemination into the food production system from environmental sources is not available. However, in some cases, the type of strain or sequence type, as well as detailed genetic characterisation of ARGs may suggest its origin.

Plant-based food production sector

- Exposure to faecal material either through specific agricultural practices (i.e. organic fertilisation with manure) or accident (e.g. irrigation with faecally contaminated surface water, contamination with runoff from fields fertilised with faecal material) is a major source of contamination. Other potential sources include soil, dust (e.g. originating from neighbouring farms), farm animals, wildlife, arthropods, workers, contaminated equipment and process water.
- Reclaimed water, increasingly used as irrigation water in arid and semi-arid areas, might represent a risk of contamination with ARB and ARGs.
- Protected crops grown in low tunnels and greenhouses are less exposed to contamination by the external environment, although irrigation water, manure and workers will still be potential sources.

Terrestrial animal production sector

Preharvest

- Feed might be contaminated by a range of resistant bacteria, which will not be eliminated by most heat or chemical treatments that are currently used, as shown for non-resistant pathogens. Bacteria such as *Salmonella* can multiply during the cooling of heat-treated feed. Improperly stored feed can also become contaminated at farm level, e.g. by farm equipment, wild birds and rodents.
- Workers and visitors, as well as equipment, might be a source of AMR. In general, most studies investigated transmission from animals to workers. Limited evidence shows transmission to occur in the other direction.
- Rodents and arthropods as well as wildlife and companion animals might serve as sources of AMR. However, the impact of these sources on AMR burden at farm level is unclear.
- Bedding materials, water, air/dust, other animal species on site than the species that is bred, might all be potential sources of ARB or ARG, but neither a causal relationship nor the extent of transmission from these sources to animals has been investigated.
- Animals kept outdoors (e.g. outdoor cattle, poultry or pig farms) will be more exposed to ARB and ARG from exterior sources such as pastures, soil, water sources, wildlife or other domestic animal species as compared those in closed facilities.
- Feeding waste milk to veal calves at early stage might be a source of ARB and ARG and a potential source of antimicrobial residues that can impact the microbiota at the development stage.

Post-harvest

- Contact with contaminated crates or vehicles during transport and lairage within the slaughterhouse) are possible transmission routes, as these environments are heavily contaminated with food-borne pathogens which may carry ARGs.
- Slaughterhouses become contaminated by ARB and ARG from animals, animal faeces/spilled intestinal contents and carcasses. Thereafter, raw materials, machinery/equipment, workers or aerosols may serve as sources and transmission routes of ARB.
- Meat processing plants acquire contamination from residual bacteria on carcasses after slaughter. Some bacteria, such as *Salmonella* and *Listeria* spp., can persist on processing equipment, surfaces and other environmental niches, such as drains, in slaughter and processing plants.

Aquaculture

- Water, and associated sediment, is a major route for disseminating ARB, some of which are opportunistic pathogens of fish and humans, to finfish and shellfish. The wider aquatic environment acts as the medium for dissemination of AMR from human and terrestrial livestock faecal waste. Feed can also be a source for ARB.
- Bivalve molluscs, including mussels and oysters, filter large volumes of water and can concentrate particulate material and pathogens and are therefore uniquely vulnerable to bacterial contamination of river and coastal water, including ARB.
- Wildlife is an additional potential source of AMR contamination in aquaculture systems.
- Introduction of AMR to aquaculture products during post-harvest processing may occur through contamination by workers, water, ice and equipment.

3.2. Public health relevance of antimicrobial-resistant bacteria and resistance genes in food-producing environments

In order to answer ToR2 (details on methodology in Section 2), criteria used for ARB/ARG risk prioritisation, their applicability to the food sector and value as predictive tools for future resistance problems, are presented in Section 3.2.1. The AMR threats considered of highest priority, based on the EMA Antimicrobial Advice Ad Hoc Expert Group (AMEG) list (EMA/CVMP/CHMP, 2020) and expert opinion, are presented in Section 3.2.2. Further differentiation of those AMR threats in the context of their application to food production environments is shown in Section 3.2.3, and additional detailed information is included in Appendix F. The main factors influencing their occurrence and persistence in food-producing systems and food were also addressed in Section 3.2.4.

3.2.1. Approaches for prioritisation of antimicrobial resistance

The ability to cause disease and their impact in terms of incidence, severity, duration and mortality in a population is usually assessed to quantify the bacterial disease burden and to subsequently establish public health priority - based interventions. Acquisition of AMR may increase the disease burden of a pathogen, due to the enhancement of the probability of causing infection and the severity of the disease, especially in cases of treatment failure (Mølbak, 2005). ARB have a selective advantage in patients treated with antimicrobials for other purposes. For instance, the development of illness by antimicrobial-resistant non-typhoidal *Salmonella* colonising the intestines is frequently observed in patients treated with antimicrobials for any medical condition. Moreover, ARB strains may easily prevail in settings where antimicrobial selective pressure is high, such as certain farms or hospitals and increased shedding and transmission of an ARB is also likely to occur as a result of the use of antimicrobial agents to which the pathogen is resistant. ARB can also promote dissemination of genes encoding for resistances that are located on mobile genetic elements to other bacteria. Delays in bacterial eradication due to acquired resistance to antimicrobials used in empirical treatment or unavailability of therapeutic options may adversely impact morbidity and mortality rates (Kumar et al., 2006; Paul et al., 2010; Seymour et al., 2017). Additionally, resistance acquisition by bacteria can be associated with virulence enhancement resulting in increased risk of invasive infections, hospitalisation and death. Co-selection of virulence traits (e.g. by location of virulence and resistance in the same plasmid), upregulation of virulence genes or improved fitness of the bacteria are possible mechanisms underlying the enhanced virulence of clones that have acquired an ARG (Mølbak, 2005; Pan et al., 2020).

The prioritisation of ARB has traditionally focused on a combination of AMR phenotype(s) and host identity. For certain priority resistance phenotypes, genotypic characterisation has been used to further define the public health priority based on resistance gene identity, followed by evidence of mobility and characterisation of MGEs. Differentiation of pathogenicity potential of strains within bacterial species is not considered within these ARB classifications, an aspect that is important to comprehensively and accurately classify ARB threats in the food chain.

Recently, ARB prioritisation based on antimicrobial-resistant bacteria likelihood and severity of infections, measured in terms of disability-adjusted life years (DALYs) has been used to estimate the public health burden caused by these threats (Cassini et al., 2019). DALYs combine the likelihood (e.g. the number of cases) and the burden due to both death and morbidity into one index. E.g. a mild disease (i.e. low DALY/case) caused by a highly prevalent hazard may have a lower total DALY than a severe disease (i.e. high DALY/case) that is caused by a hazard that is rare. An estimation of the health burden, in DALYs, for 16 AMR–bacterium combinations causing infections in European countries indicates that it is substantial when comparing with other infectious diseases (e.g. influenza, tuberculosis or HIV), and increasing since 2007 (Cassini et al., 2019). Such estimations are not available to quantify the health burden of food-borne ARB pathogens or the ones caused indirectly by non-pathogens which may act as donors of ARGs.

This clinical perspective focuses on the current problem and arguably does not encourage consideration of the complex evolutionary and ecological trajectories that lead to the association of 'new' uncharacterised ARGs with previously susceptible pathogens. Studies within human, animal and environmental microbiomes provide limited information as relatively few studies consider the resistome in its entirety, with most focusing on resistance in a small number of organisms such as *E. coli*. Attempts have been made to consider AMR in its entirety and to classify relative risk to public health posed by different ARGs in different contexts. Even in the absence of data on host identity, this approach can allow some understanding of risk posed by specific genes within genomes or metagenomes. A conceptual framework for considering prioritisation of ARG was proposed by Martínez et al. (2015) entitled 'What is a resistance gene? Overall, the key criteria in the ranking scheme are the burden of evidence that a given ARG is compromising or could comprise drugs used in treating infections in humans, and some measure for the potential of HGT through association (or not) with MGEs. There are questions regarding this ranking system, particularly around the absence of risk differentiation within the highest risk class and the relative risk attributed to antimicrobials still in development (which presumably may become of very high importance) and to genes not located on MGEs (which can be readily mobilised from the chromosome by a multitude of mechanisms).

Recently Zhang et al. (2019b) proposed a risk ranking scheme based on three criteria: 1) enrichment in human-associated environments (human-associated enrichment), 2) gene mobility and 3) presence/absence in ESKAPE[35] pathogens (host pathogenicity). The scheme is very simple, employs the two key criteria of Martínez et al. (2015), namely evidence of HGT and association with human pathogens, but more heavily weights anthropogenic effects on the environmental resistome. In the Zhang et al. framework, criterion 1 emphasises the importance of human-impacted environments that can receive inputs of human or animal faeces, therefore mixing ARGs enriched in humans or farm animals, and their associated MGEs with the environmental bacteria (Karkman et al., 2019; Peters and Zitomer, 2021). Furthermore, ARGs can be enriched in environments that are polluted by antimicrobials or other selective or co-selective chemicals such as effluents from pharmaceutical manufacturing, mining or smelting (Milaković et al., 2020; Corella et al., 2021).

Although these frameworks emphasise risks associated with ARGs that are of human health concern, they are not predictive for the acquisition of cryptic ARGs that could be recruited from the environmental resistome under favourable circumstances and through evolutionary phenomena that are largely unknown (D'Costa et al., 2006; Wright, 2007). It is important to recognise that phenomena that cannot be predicted, including 'repurposing' of genes with natural functions other than resistance can confer *de novo* phenotypic resistance. Modification of 'proto' ARGs through mutation into functional ARGs, and their subsequent HGT, may create the next future ARG problem. The focus on genes associated with MGEs also ignores the fact that potentially all genes can be mobilised. Key chromosomally located genes in related species of *Kluyvera,* e.g. were mobilised giving rise to different $bla_{\text{CTX-M}}$ groups that have appeared in Gram-negative pathogens and are now responsible for substantial treatment failure (Humeniuk et al., 2002).

3.2.2. Definition of antimicrobial-resistant bacteria and resistance genes of highest priority for public health in food-producing environments

The aim of ToR2 was to identify ARB and ARG of highest priority for public health among those that might be transmitted to food chain through the routes identified by ToR1. As indicated in Section 2.2 (Methodologies), the first step consisted of the definition of the ARB and ARG of highest priority for public health.

The exercise was informed by available international documents on prioritisation of antimicrobial agents and resistant pathogens (WHO, 2019a,b; EMA, 2020). In particular, the EMA list of bacteria causing human infections against which there are few treatment alternatives [see Table A1, EMA/CVMP/CHMP, 2020)] was selected to further define ARB of highest Public Health relevance in the food environmental context.

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[35] *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.

From the EMA (2020) list, microorganisms that might be acquired by humans through food-borne exposure or exposure to food production environments were identified, and subsequently separated into two groups, based on further considerations of pathogenicity potential and profile of AMR (Table 6). Species, serotypes or lineages associated with infection and resistant to antimicrobials of choice for the treatment of serious bacterial infections (e.g. macrolides and fluoroquinolones for *Campylobacter* infections) or to last resort antibiotics (WHO, 2019a,b), were included in **Group 1** and organisms without recognised potential of causing infection, commensal or environmental bacteria, with mobile resistance genes to last resort antibiotics (WHO, 2019a,b), were included in **Group 2**.

ARGs horizontally transferable between bacterial cells and conferring resistance to last resort antimicrobials (WHO, 2019a,b; antimicrobials mentioned in Table 6) are considered to be of the highest priority. These genes are included in rank I of the Zhang et al. (2019b) resistance genes scheme mentioned in Section 3.2.1.

Table 6: Antimicrobial-resistant bacteria of highest priority for public health in food-producing environments

	ARB	Justification for inclusion
Group 1	Non-typhoidal *Salmonella enterica* serovars resistant to 3rd-GCs, carbapenems or fluoroquinolones.	• Invasive infections (invasive non-typhoidal *Salmonella*; INTS) caused by this food-borne pathogen require treatment with antimicrobials targeting intracellular sites of infection, e.g. within the reticuloendothelial system or gallbladder. • Due to the common resistance to aminopenicillins, the 3rd-GCs, fluoroquinolones and carbapenems are the preferred options for those infections, which occur with an incidence of ~ 1.1 per 100,000 population in Europe (Non-Typhoidal Salmonella Invasive Disease Collaborators, 2019). • *Salmonella* Enteritidis, the monophasic variant of *S*. Typhimurium, *S.* Typhimurium are common in INTS, although *Salmonella* Dublin, Choleraesuis, Heidelberg, Napoli and Virchow are also among those most likely to cause bacteraemia (Jones et al., 2008; Mastrorilli et al., 2020). • A large proportion of *S*. Enteritidis lineages, and those of serovars commonly found associated with INTS, show resistance to fluoroquinolones or to 3rd-GCs. Resistance to carbapenems has also been occasionally observed in *S*. Infantis and *S*. Kentucky, serotypes that can also sporadically cause INTS (de Curraize et al., 2017).
	Campylobacter spp. resistant to macrolides, fluoroquinolones, aminoglycosides or carbapenems.	• Antibiotic treatment is required for invasive *Campylobacter* infections, a rarely reported condition (Kaakoush et al., 2015). Although mostly caused by *C. jejuni, C. coli, C. fetus* and *C. lari* have also been associated with this zoonosis. • Resistance to macrolides and fluoroquinolones, the common therapeutic options, is frequently observed.
	Enterobacterales other than *Salmonella* spp. resistant to 3rd-, 4th- and 5th-GCs, carbapenems, colistin, plazomicin, fluoroquinolones or glycylcyclines.	• *E. coli*, *K. pneumoniae* and *Enterobacter* spp., common causes of serious infections, are increasingly presenting multidrug resistance profiles including to last resort antibiotics. • Those resistant human infections have been often caused by particular *E. coli* (e.g. ST131 H30, ST10, ST38, ST69, ST393, ST405, ST410, ST648) or *K. pneumoniae* (e.g. ST258, ST307, ST11, ST15, ST101, ST147) lineages/sub-lineages.

	ARB	Justification for inclusion
		• Among the *Enterobacter* spp., certain carbapenem-resistant *E. hormaechei* lineages have been increasingly identified in human infections (e.g. ST171, ST78) (Guzmán et al., 2019; Gou et al., 2020; Tavovoschi et al., 2020). • There is evidence of resistant ExPEC of food origin causing human infections, although the burden of disease associated with this origin is still controversial (Mughini-Gras et al., 2019). • Recent studies have focused on *K. pneumoniae,* but there remains little information regarding the role of food-producing animals and food products on the transmission of this pathogen to humans. Even less information is available for *E. hormaechei.*
	S. aureus resistant to methicillin, 5th-generation cephalosporins, glycopeptides, oxazolidinones, lipopeptides or glycylcyclines.	• The mean percentage of methicillin-resistant *S. aureus* (MRSA) causing human invasive infections in the EU was 15.5% in 2019, ranging from 1.1% to 46.7% among member states (ECDC, 2020). • MRSA with additional resistance to other antimicrobial groups is common and occurs in a diversity of types of MRSA, including MRSA associated with healthcare or community settings or livestock. • Vancomycin, ceftaroline, ceftobiprole, linezolid, daptomycin or tigecycline are used as alternative antimicrobials in human settings and contamination of food system environments with MRSA presenting resistance to these antimicrobials may occur.
	Enterococcus faecium and *E. faecalis* resistant to glycopeptides or oxazolidinones, lipopetides or glycylcyclines.	• Hospital-associated infections by *E. faecium* (HA-*E. faecium*) and *E. faecalis* often require treatment with glycopeptides and oxazolidinones due to the intrinsic and acquired resistance presented by those species. • HA-*E. faecium* comprises a specialised subpopulation of *E. faecium* (clade A; nowadays mainly dominated by ST78-related strains such as ST80, ST117 and ST203) enriched in virulence and resistance genes (Freitas et al., 2018). • These multidrug-resistant clones frequently carry vancomycin resistance genes on plasmids and, with increasing frequency, also point mutations or transferable genes encoding linezolid resistance (Egan et al., 2020). • Resistant *E. faecalis* lineages causing infections are diverse and reflect the generalist lifestyle of this organism. However, they have mainly been associated with particular subpopulations (e.g. ST6, ST9, ST28, ST40, ST87, ST103) that are enriched in antimicrobial resistance and virulence genes (Guzmán Prieto et al., 2016; Raven et al., 2016).
	Acinetobacter baumannii and *Pseudomonas aeruginosa* resistant to carbapenems and colistin.	• *A. baumannii* multi-drug resistant strains causing hospital infections predominantly belong to particular lineages (e.g. CC231, CC208, CC447, ST944 and ST950) with evidence of enhanced virulence and resistance (Silva et al., 2021). • Particular clones (e.g. ST111, ST175, ST244 and ST253) of *P. aeruginosa* presenting MDR and plasmid-encoded carbapenemases with enhanced virulence also often cause human infections (Gaiarsa et al., 2019). These human clinical lineages have

	ARB	Justification for inclusion
		not been as far as we know reported in food products.
Group 2	*Enterobacterales, Pseudomonas* spp., *Acinetobacter* spp., *Aeromonas* spp. and *Vibrio* spp. with mobile resistance genes to last resort antibiotics	• Environmental or commensal bacteria could act as donor of resistance genes to Gram negative pathogenic bacteria. Mobile genes encoding carbapenemases, ESBL/AmpC cephalosporinases, 16S rRNA methylases or resistance to glycylcycline, polymixines and fluoroquinolones are of highest relevance. • The human disease burden resulting from antimicrobial resistant indigenous aquatic or fish bacteria has been insufficiently studied. Moreover, insufficient information on the lineages or serotypes able to cause infection and on the AMR profiles of aquatic and fish indigenous bacteria belonging to *Vibrio parahaemolyticus, V. vulnificus, Aeromonas or non-aeruginosa Pseudomonas* species precludes their current inclusion in Group 1.
	Enterococcus spp. with mobile resistance genes to last resort antibiotics	• These commensal bacteria could act as donor of genes conferring resistance to last resort antimicrobials to other Gram-positive pathogenic bacteria. • Mobile resistance genes for isoxazolidinones and vancomycin are of highest relevance.
	Staphylococcus spp. with mobile resistance genes to last resort antibiotics	• These commensal bacteria could act as donor of genes conferring resistance to last resort antimicrobials to other Gram-positive pathogenic bacteria. • Mobile genes encoding resistance to methicillin (e.g. *mecB*, *mecC* and *mecA*) isoxazolidinones are of highest relevance.

3rd GCs: 3rd-generation cephalosporins.

3.2.3. Antimicrobial-resistant bacteria and resistance genes of high public health relevance in environmental sources of food-producing sectors

The information collected, as described in Sections 2.1 and 3.2.2, on public health highly relevant bacteria and genes was summarised for each food-producing sector and included in Tables 7–11. A more detailed description of some relevant findings is presented in Appendix F.

Overall, data on AMR in food production environments is limited and the studies have not been systematically conducted, have used different sampling and testing strategies and are mainly focused on a limited range of organisms and resistance profiles. Reports focus particularly on ESBL/AmpC related plasmidic genes such as *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{CMY-2}}$ in *E. coli* and *Salmonella* and fluoroquinolone-resistant *Campylobacter* or *E. coli* associated with chromosomal mutations in *gyr*A, vancomycin resistant (*van*A) enterococci or methicillin-resistant *Staphylococcus aureus* (MRSA).

3.2.3.1. Plant-based food production sector

Examples of ARB and ARG found in the different potential AMR environmental sources identified in the plant-based production sector are presented in Table 7. More detailed information can be found in Appendix F.1. Supporting references are included in the tables and appendix text.

Bacterial resistance to highly important antibiotics, including to extended spectrum cephalosporins, fluoroquinolones, carbapenems, colistin and glycopeptides, was identified in different environmental sources.

Resistance to highly important antibiotics due to mobile resistance genes in commensal or environmental isolates have been described. Particularly, MDR *E. coli* with resistance to extended spectrum cephalosporins has been described in manure from various animal species (poultry, pigs and dairy cattle) and irrigation water. Moreover, transferable resistance to colistin has also been reported in bacteria from pig manure and carbapenem resistant *E. coli* was reported in reused water, from effluent of sewage treatment plants, together with carbapenem-resistant *K. pneumoniae* and *Citrobacter freundii*. Descriptions of MDR and vancomycin-resistant *E. faecium* and ciprofloxacin-resistant *E. faecalis* and carbapenem-resistant *A. baumannii* in pig manure are also presented.

Table 7: Distribution of antimicrobial resistant bacteria and resistance genes in potential environmental sources of contamination of crops produced in open systems: examples based on European literature

Main sources	Bacteria [antimicrobial resistance profiles and genes](a)	Detection of Group 1 ARB/highest relevant ARG(d),(e)/comments	Factors influencing persistence and occurrence	Supporting references(f)
Poultry manure	*E. coli* [3rd-GCs; ESBL-phenotype]	**?/+**	Antimicrobial residues in the manure, competing microflora, composting conditions (e.g. temperature, moisture)	Graham et al. (2009), Hering et al. (2016)
Pig manure	*E. coli* [MDR; 3rd-GCs; COL; *aadA1, aadA2,* $\underline{bla_{\text{CTX-M-1}}}$*, cmlA1-like,* <u>*mcr-1*</u>*, mph*(A)*, sul3, tet*(A)*-like*]; *E. coli* [3rd-GCs; $\underline{bla_{\text{CTX-M-1}}}$; $\underline{bla_{\text{CTX-M-15}}}$, $\underline{bla_{\text{CTX-M-9}}}$](b)	**?/+** Colistin resistance gene in IncX4 plasmid very similar to one of human clinical origin.	Resistance to multiple antimicrobials including to those; AMR phenotypes usually horizontally transferable	García-Cobos et al. (2015), Guenther et al. (2017),
	E. faecalis [MDR; *tet*(M), *tet*(L), *erm*(B); *aac(6′)-Ie-aph(2′)- Ia*]	**?/+** Numerous AMR phenotypes transferable by conjugation.	Tolerance to Cu with co-transference of resistance to several antimicrobials (e.g. vancomycin) under Cu selective pressure	Novais et al. (2013), Silveira et al. (2013)
	E. faecium [VAN; <u>*vanA*</u>, *tet*(M), *tet*(L), *erm*(B)](b)	**+/+** Numerous AMR phenotypes transferable by conjugation including VAN-R		
	A. baumanii [CARBA; $\underline{bla_{\text{OXA-23}}}$]	**?/+**		Hrenović et al. (2019)
Dairy or beef manure	ESBL-*E.coli* [3rd-GCs; $\underline{bla_{\text{CTX-M-14}}}$; $\underline{bla_{\text{CTX-M-15}}}$; $\underline{bla_{\text{CTX-M-27}}}$](b)	**+/+** ST10 lineage in slurry and clinical isolates		Day et al. (2019)
Irrigation water; natural	*E. coli* [MDR; 3rd-GCs; $\underline{bla_{\text{CTX-M-27}}}$, *strA/strB, aadA5,, mph*(A)*, sul1, sul2, tet*(A)*, dfrA17*]; [*strA/strB, aphA1,* $bla_{\text{TEM-1B}}$*, sul2*]; [$\underline{bla_{\text{CTX-M-14b}}}$*, aadA1, dfrA1*]; [$bla_{\text{TEM-1}}$]; [*tet*(B)*, aadA1, dfrA1*](b)	**+/+** ARGs on plasmids from varied incompatibility groups IncY; IncFIA; IncFIB; IncQ1. ST10 and other lineages associated with human infections.		Araújo et al. (2017), Gekenidis et al. (2018)
Irrigation water; reclaimed	*E. coli* [CARBA; $\underline{bla_{\text{OXA-48}}}$, $\underline{bla_{\text{NDM-5}}}$, $\underline{bla_{\text{VIM-1}}}$] *K. pneumoniae* [CARBA; $\underline{bla_{\text{OXA-48}}}$, $\underline{bla_{\text{NDM-9}}}$, $\underline{bla_{\text{KPC-2}}}$] *C. freundii* [CARBA; $\underline{bla_{\text{OXA-48}}}$]	**?/+** Effluent from Basel or Warsaw sewage treatment plants. Potential for transmission of carbapenemases common in clinical isolates through crops if used for irrigation without further treatment.		Zurfluh et al. (2017)

Main sources	Bacteria [antimicrobial resistance profiles and genes](a)	Detection of Group 1 ARB/highest relevant ARG(d),(e)/comments	Factors influencing persistence and occurrence	Supporting references(f)
Dust	MRSA [*mecA*, *tet*(W)]	**+/+** Pig barn dust; potential for crop exposure if released.		de Rooij et al. (2019)
	E. faecium [CIP]	**–/–** Pig and poultry barns.		Liu et al. (2018)
	E. coli [3rd-GCs; *bla*$_{\text{TEM-1}}$, *bla*$_{\text{CTX-M}}$]	**?/+**		Laube et al. (2014)
Soil	[*bla*$_{\text{OXA-48}}$, *bla*$_{\text{TEM}}$, *tet*(M)](c)	**ND/+**		Cerqueira et al. (2019a,b)

(a): Multidrug (MDR) and resistance phenotypes to last resort antibiotics in capital letters; acquired resistance genes italicised; genes conferring resistance to last resort antibiotics are underlined.
(b): Diverse phenotypes and/or different resistance-determinant combinations.
(c): Resistance genes detected by metagenomic or other approaches.
(d): Group 1 ARB (according to definition in Table 6) were present (+), absent (–) or no information provided to indicate their presence (?)/ARG of highest relevance (according to definition in Section 3.2.2) were present (+), absent (–) or no information provided to indicate their presence (?).
(e): Horizontal transferability of resistance is assumed for resistance phenotypes or genes usually acquired by this process (e.g. *bla*$_{\text{CTX-M}}$ and *mcr* genes).
(f): Source of the data presented in 'Bacteria [antimicrobial resistance profiles and genes]' column and in the categorisation as Group 1 ARB/highest relevant ARG.
Antimicrobials: CARBA – carbapenems; 3rd-GCs – third-generation cephalosporins; COL – colistin; VAN – vancomycin; Other acronyms: ND – not determined; Cu – Copper.
ESBL: extended spectrum beta-lactamase; MRSA: methicillin-resistant *Staphylococcus aureus*; ST: multi-locus sequence type.

3.2.3.2. Poultry production sector

Examples of ARB and ARG found in the different potential AMR environmental sources identified in the poultry production sector are presented in Table 8. More detailed information can be found in Appendix F.2.1. Supporting references are included in the tables and appendix text.

A wide variety of ARB and ARGs have been reported from the poultry intestinal tract and, to a lesser extent, the poultry farm environment. Bacterial resistance to highly important antibiotics was identified in different environmental sources, and particularly to extended spectrum cephalosporins and fluoroquinolones. Resistance to vancomycin has been more rarely reported. Antimicrobial resistances more recently studied include to carbapenems, colistin, oxazolidinones or plazomicin, particularly described in wild birds and abattoirs waste.

MDR *Salmonella* resistant to extended spectrum cephalosporins or fluoroquinolones and *Campylobacter* strains with high level fluoroquinolone resistance are of the highest public health relevance and have been described in different environmental sources. Also of highest public health relevance are the extended spectrum cephalosporins-resistant Enterobacterales strains associated with human infections described (e.g. in slaughterhouse environments).

Resistance to highly important antibiotics due to mobile resistance genes in commensal isolates has been extensively described. MDR *E. coli* and other Enterobacterales with ESBL/AmpC plasmid-mediated genes and strains with high level fluoroquinolone resistance are commonly found in poultry environments (e.g. rats, flies, wild animals, manure/litter). Some strains, namely from wastewaters, also have transferable (*mcr*-mediated) colistin resistance. Transferable resistance to carbapenems or plazomicin (e.g. *armA*) has also been occasionally reported in different Gram-negative bacteria.

One additional concern is the spread of enterococci or staphylococci resistant to oxazolidinones, particularly when carrying transferable oxazolidinone resistance genes as their acquisition usually also confers resistance to phenicols and tetracyclines, common veterinary medicines which might enhance the burden of these ARGs in enterococci and staphylococci. These AMR threats have seldom been described in poultry environments in European countries.

Table 8: Distribution of antimicrobial-resistant bacteria and resistance genes in potential environmental sources of contamination of poultry farms and processing facilities: examples based on European literature

Main sources	Bacteria [antimicrobial resistance profiles and genes](a)	Detection of Group 1 ARB/highest relevant ARG(d),(e)/comments	Factors influencing persistence and occurrence	Supporting references
Carrier chicks from primary (elite/pedigree/GGP) breeding flocks	*E. coli* (MDR, 3rd-GCs and FQ; $\underline{bla_{\text{CTX-M-1}}}$; $\underline{bla_{\text{CMY-2}}}$, $\underline{bla_{\text{SHV-12}}}$)(b)	**?/+**	Ongoing AMU, inadequate cleaning, disinfection and pest control; vertical dissemination, persistence and clonal expansion	Persoons et al. (2011), Dame-Korevaar et al. (2017), Nilsson et al. (2020)
	E. coli [FQ](b)	**?/–**		Kaspersen et al. (2020a)
	Salmonella enterica [MDR; FQ](b)	**+/?** Turkey hatcheries		Mueller-Doblies et al. (2013)
Survival between flocks: persistence within the environment of poultry houses (failed cleaning and disinfection)	Multiple ARB (*S. aureus*, *Enterococcus* spp.)	**?/?**	Ongoing AMU, inadequate cleaning, disinfection and pest control. Poor internal and external biosecurity. Complex reservoir of ARB that can be selected if antimicrobial treatment is used. Perpetuation of infection/ carriage in poultry and contamination of poultry products.	Brooks et al. (2010)
	E. coli [FQ]	**?/?**		Taylor et al. (2016)
	Enterococcus faecium [MDR; VAN].	**?/+**		Nilsson et al. (2009), Jansson et al. (2012)
Rodents	*S.* Infantis [MDR]	–/–	Untidy farms that are attractive to rodents, lack of proofing, poor rodent monitoring and control programmes, rodenticide resistance.	Nógrády et al. (2008)
	E. coli [MDR; 3rd-GCs](b)	**?/+**		Himsworth et al. (2016)

Main sources	Bacteria [antimicrobial resistance profiles and genes][(a)]	Detection of Group 1 ARB/highest relevant ARG[(d),(e)]/comments	Factors influencing persistence and occurrence	Supporting references
Wildlife (wild birds)			Access of wild birds to human or animal faecal waste, lack of suitable farm biosecurity. Potential sources with occasional observations of phage types/genotypes of *S*. Typhimurium that are adapted to certain wild bird species in poultry production.	
	Salmonella Corvallis [CARBA; *bla*$_{\text{NDM-1}}$, *bla*$_{\text{CMY-16}}$, *dfrA1-aadA5* or *aacA4*, *floR*, *tet*(A), *strA/B*, *sul1*, *sul2*]	**+/+** CRE rarely reported in EU in food animals or wildlife, but more common elsewhere. In black kite.		Fischer et al. (2013), Köck et al. (2018)
	E. coli/*Klebsiella* sp. [3rd-GCs; *bla*$_{\text{CMY-1}}$, *bla*$_{\text{CMY-2}}$, *bla*$_{\text{SHV-12}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-3}}$, *bla*$_{\text{SHV-11}}$, *bla*$_{\text{SHV-167}}$][(b)]	**?/+** In raptors		Darwich et al. (2019)
	Campylobacter jejuni [MDR]	**+/–** In corvids		Söderlund et al. (2019)
	E. coli [MDR; 3rd-GCs; *bla*$_{\text{CTX-M-55}}$, *arr2*, *acc*(3)-IId, *aad22*, *aph*(3′)-Ia, *aph* (3″)-Id, *aph*(6)-Id, *cmlA1, dfr14, floR, mefB, mdfA, mphA, qnrS1*, *sul3*, *tetA*][(b)]	**?/+** In corvids		Söderlund et al. (2019)
	E. coli [FQ; 3rd-GCs; *bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-32}}$, *bla*$_{\text{CTX-M-65}}$, *bla*$_{\text{SHV-12}}$; *bla*$_{\text{CTX-M-3}}$ and *bla*$_{\text{CTX-M-55}}$; *qnrS* and *qnrB aac(6′)-Ib-cr*][(b)]	**?/+** In vultures		Blanco et al. (2020)
	E. faecium [MDR; LIN]	**?/–** Screening only for *cfr* gene In vultures		Blanco et al. (2020)

Main sources	Bacteria [antimicrobial resistance profiles and genes]$^{(a)}$	Detection of Group 1 ARB/highest relevant ARG$^{(d),(e)}$/comments	Factors influencing persistence and occurrence	Supporting references
Arthropods	*E. coli* and other Enterobacteriaceae [MDR; 3rd-GCs; *bla*$_{\text{SHV-12}}$, *bla*$_{\text{TEM-52}}$, *bla*$_{\text{CTX-M-1}}$; *bla*$_{\text{CTX-M-9}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CMY-2}}$; *floR*; *qnrS*; *aac(3)-IIa*, *strA/strB*; *sul2*; *tet* (A)]$^{(b)}$	**?/+** In flies.	Access of flies to human or animal faecal waste. Wet litter/manure and dead bird storage attracts flies. Lack of timely interventions. Litter removed from the house contains these vectors and they can disseminate to other poultry flocks if litter is not completely removed from the farm.	Blaak et al. (2014), Solà-Ginés et al. (2015), Poudel et al. (2019)
Workers, visitors	*E. coli* [MDR]$^{(b)}$	**?/–**	Contamination of poultry farm workers higher in turkey > broiler > layer	Van den Bogaard et al. (2001)
	Salmonella Kentucky [MDR, FQ]	**+/–**		Guillon et al. (2013)
Equipment (transport crates)	*Campylobacter* sp. [MDR, FQ]	**+/–**	Inadequate crate wash machines and training of bird catching teams. Poor disinfection of vehicles that enter poultry houses during catching and chick delivery	Peyrat et al. (2008)
Water supplies	*E. coli* [MDR; FQ]$^{(b)}$	**?/?**	Use of non-municipal water or ineffective water treatments. Persistence within water pipes as biofilm or protected within protozoa.	Coleman et al. (2013)
Feed			Bacterial contamination of growing and stored crops is common but there is little information on AMR in feeding stuffs and nothing on colonisation after ingestion apart from *Salmonella.* Wild animal exposure during growing or storage of feed ingredients, particularly those imported from high-risk countries or produced on livestock farms.	
	E. coli [MDR; FQ]$^{(b)}$	**?/?**		da Costa et al. (2007)
	Salmonella [MDR]$^{(b)}$	–/–		Wasyl and Hoszowski (2004)

Main sources	Bacteria [antimicrobial resistance profiles and genes]$^{(a)}$	Detection of Group 1 ARB/highest relevant ARG$^{(d),(e)}$/comments	Factors influencing persistence and occurrence	Supporting references
	E. hirae/E. durans/E. faecalis/E. faecium [MDR; FQ; VAN]$^{(b)}$	**?/+**		da Costa et al. (2007)
Manure/litter			Incomplete removal of manure or storage close to poultry houses leading to run off and arthropod pests re-entering cleaned housing	
	E. coli [MDR; 3rd-GCs; $\mathit{bla}_{\text{CMY}}$; $\mathit{bla}_{\text{CTX-M}}$; $\mathit{bla}_{\text{TEM-52}}$; $\mathit{bla}_{\text{SHV-12}}$; $\mathit{bla}_{\text{TEM-1}}$]$^{(b)}$	**?/+**		Laube et al. (2014)
	E. coli [MDR; *catI; catII, qnrS, tet*(A); *tet* (M), *sul1, sul2, sul3; dfrIa*]	**?/+**		Amador et al. (2019)
	[$\mathit{bla}_{\text{TEM}}$, $\text{bla}_{\text{CTX-M}}$, *mecA*]$^{(c)}$	**ND/+**		Colomer-Lluch et al. (2011)
Dust/Air			Intensive poultry production produces large quantities of contaminated dust, originating from the faeces and integument of birds, that can spread for long distances. Dust can be blown between farms, between different groups of animals on farms and can persist in poorly cleaned houses. Some bacteria that can be MDR such as *Salmonella, E. coli* (CIP/3rd-GCs resistant) and MRSA can survive for years in dust.	
	E. coli [MDR; FQ; 3rd-CGs; $\mathit{bla}_{\text{CMY}}$; $\mathit{bla}_{\text{TEM-1}}$; $\mathit{bla}_{\text{CTX-M}}$, $\mathit{bla}_{\text{SHV-12}}$, $\mathit{bla}_{\text{TEM-52}}$, $\mathit{bla}_{\text{CTX-M-2}}$, $\mathit{bla}_{\text{CTX-M-14}}$, $\mathit{bla}_{\text{CTX-M-15}}$, $\mathit{bla}_{\text{CTX-M-27}}$]$^{(b)}$	**?/+** Dust		Blaak et al. (2015), Schulz et al. (2016), de Rooij et al. (2019), Schulz et al. (2019)
	MRSA [MDR; *mecA*]	**?/+** Air		Friese et al. (2013), de Rooij et al. (2019)

Main sources	Bacteria [antimicrobial resistance profiles and genes][(a)]	Detection of Group 1 ARB/highest relevant ARG[(d),(e)]/comments	Factors influencing persistence and occurrence	Supporting references
Wastewater: wash/surface water			There is little data on surface water associated with poultry farms apart from in far Eastern countries. It is clear that this can become contaminated via dust, litter or washing of poultry houses and accumulation of surface water is attractive to wild birds. Poor drainage and water storage facilities, as well as washing houses during wet weather exacerbate problems with incomplete removal of wash water	
	E. coli (MDR; 3rd-GCs; $\mathit{bla}_{\text{SHV-12}}$, and $\mathit{bla}_{\text{TEM-52}}$, $\mathit{bla}_{\text{CTX-M-2}}$, $\mathit{bla}_{\text{CTX-M-14}}$, $\mathit{bla}_{\text{CTX-M-15}}$, $\mathit{bla}_{\text{CTX-M-27}}$)[(b)]	**?/+**		Blaak et al. (2015)
	S. Infantis [MDR; $\mathit{bla}_{\text{TEM-1}}$, *strA/B*, *sul1*, *sul2*, *tet*(B), *catA1*, *catB3*, *aphA1* and *aadA4*]	**–/–** class 1 integrons, conjugative plasmids	Co-resistance	Dionisi et al. (2011)
	[$\mathit{bla}_{\text{DHA}}$, $\mathit{bla}_{\text{NDM}}$, $\mathit{bla}_{\text{TEM}}$, $\mathit{bla}_{\mathit{CMY-2}}$, *tet*(M), *mcr-1*][(c)]	**ND/+**		Hubbard et al. (2020)
Soil			Soil can become contaminated as a result of emissions from poultry house, run-off and application of litter/manure or defaecation by free-ranging poultry. The role of soil as a source of infection of poultry is unclear. Stocking density and manure application rates, plus the presence of antimicrobials or heavy metals in applied manure influence the occurrence, level and persistence of ARB in soil. Persistence varies with the type of soil and associated microbiota and worm populations. Persistence at	

Main sources	Bacteria [antimicrobial resistance profiles and genes](a)	Detection of Group 1 ARB/highest relevant ARG(d),(e)/comments	Factors influencing persistence and occurrence	Supporting references
			a similar level for 2 years after manuring ceased.	
	E. coli [MDR; 3rd-GCs; *bla*$_{\text{CTX-M-}}$, *bla*$_{\text{SHV-12}}$, and *bla*$_{\text{TEM-52}}$, *bla*$_{\text{CTX-M-2}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-27}}$](b)	**?/+**		Blaak et al. (2015)
	[*aadA*, *bla*$_{\text{TEM,}}$ *qnrS*, *sul1*, *sul2*, *str*, *tet*(A), *tet*(B), *tet*(C), *tet*(M), *tet*(Q), *tet*(W), *strpB*](c)	**ND/+**		Esperón et al. (2018)
Companion animals	*Enterococcus* spp. [VAN; *vanA*]	**?/–** In chickens, pigs, a dog and a horse on the same farm.	No evidence of specific involvement on poultry farms, although very common in certain animals. Companion animals should be prevented from accessing poultry accommodation, feed and bedding stores as part of the biosecurity program.	Bates et al. (1994)
Post-harvest: Slaughter/Abattoir/Processing plant				
Equipment/ environment (air, water, etc.)	*Arcobacter butzleri* [MDR; FQ]	**–/–**	Design of equipment that is difficult to clean, high throughput with minimal cleaning time, ineffective cleaning and disinfection programmes. There is little published data specifically on persistence of ARB on abattoir equipment. *Salmonella* has been shown to be able to survive for long periods in the slaughter equipment.	Ferreira et al. (2013)
	Campylobacter [MDR; FQ; ERY; *tet*(O), *bla*$_{\text{OXA-184}}$, *bla*$_{\text{OXA-61}}$](b)	**+/–**		García-Sánchez et al. (2019)
	E. coli [MDR, 3rd-GCs; *bla*$_{\text{CTX-M-1;}}$ *bla*$_{\text{CTX-M-15;}}$ *bla*$_{\text{CMY-2;}}$ *bla*$_{\text{SHV-12;}}$ *bla*$_{\text{TEM-1b}}$](b)	**?/+**		Gregova et al. (2014), von Tippelskirch et al. (2018)

Main sources	Bacteria [antimicrobial resistance profiles and genes](a)	Detection of Group 1 ARB/highest relevant ARG(d),(e)/comments	Factors influencing persistence and occurrence	Supporting references
	E. coli [3rd-GCs; *bla*$_{\text{CTX-M-15}}$]	**–/+** Isolates of ST361 with contamination of broiler carcasses demonstrated.		Projahn et al. (2019)
	K. pneumoniae [3rd-GCs; *bla*$_{\text{SHV-2}}$]	**+/+** Isolates of ST2762 with several virulence genes; contamination of broiler carcasses demonstrated.		Projahn et al. (2019)
	P. mirabilis [MDR; 3rd-GCs; *bla*$_{\text{CMY-2}}$]	**?/+**		von Tippelskirch et al. (2018)
	LA-MRSA/MRSA [MDR](b)	**+/+**		Mulders and et al. (2010)
Abattoir workers			Infected abattoir workers may spread infection outside the workplace and contaminate carcasses or equipment if hygiene standards are not high. As for farm workers, plus limited space for workstations and high exposure to bacterial aerosols.	
	E. coli [MDR; 3rd-GCs; *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{SHV-12}}$, *bla*$_{\text{TEM-135}}$](b)	**?/+**		Wadepohl et al. (2020)
	E. coli [MDR; *aac*(3)-*IIa*, *aph(3)*-*Ia*, *strA/B*, *sul2*, *sul3*, *bla*$_{\text{TEM}}$, *bla*$_{\text{CMY–2}}$, *mcr*-1, *tet*(A), *tet*(B), *mefB*](b)	**?/+**		Maciuca et al. (2019)
	MRSA [MDR]	**+/+**		Mulders et al. (2010)
Abattoir waste			Occurrence and level of ARB in abattoir waste depends on what is in the birds and waste treatment methods. Drains for wastewater can act as a reservoir of bacteria.	

Main sources	Bacteria [antimicrobial resistance profiles and genes](a)	Detection of Group 1 ARB/highest relevant ARG(d),(e)/comments	Factors influencing persistence and occurrence	Supporting references
	E. coli, *K. pneumoniae* and *Enterobacter* spp. [3rd-GCs; COL; *bla*$_{\text{CTX-M-1}}$; *bla*$_{\text{CTX-M-15}}$; *bla*$_{\text{SHV-2}}$; *bla*$_{\text{SHV-1}}$; *bla*$_{\text{SHV-2a}}$; *bla*$_{\text{SHV-25}}$; *bla*$_{\text{SHV-27}}$; *bla*$_{\text{SHV-28}}$; *bla*$_{\text{SHV-38}}$; *bla*$_{\text{TEM-1}}$ *bla*$_{\text{TEM-20,}}$ *bla*$_{\text{TEM-52 variants}}$, *bla*$_{\text{TEM-116}}$; *bla*$_{\text{PER}}$; *bla*$_{\text{GES}}$; *mcr-1*](b)	**?/+**		Savin et al. (2020)
	MRSA	**+/+**		Savin et al. (2020)
	Enterococcus spp. [TET; *tet*(M)]	–/–		Araújo et al. (2010)
	[*sul1*, *bla*$_{\text{TEM}}$, *bla*$_{\text{CTX-M-9,}}$ *bla*$_{\text{CTX-M-1,}}$ *mecA, armA, qnrA, qnrS*](c)	**ND/+**		Colomer-Lluch et al. (2014)

(a): Multidrug (MDR) and resistance phenotypes to last resort antibiotics in capital letters; acquired resistance genes italicised; genes conferring resistance to last resort antibiotics are underlined.

(b): Diverse phenotypes and/or different resistance-determinant combinations.

(c): Resistance genes detected by metagenomic or other approaches.

(d): Group 1 ARB (according to definition in Table 6) were present (+), absent (–) or no information provided to indicate their presence (?)/ARG of highest relevance (according to definition in Section 3.2.2) were present (+), absent (–) or no information provided to indicate their presence (?).

(e): Horizontally transferability of resistance is assumed for resistance phenotypes or genes usually acquired by this process (e.g. *bla*$_{\text{CTX-M}}$ and *mcr* genes).

(f): Source of the data presented in 'Bacteria [antimicrobial resistance profiles and genes]' column and in the categorisation as Group 1 ARB/highest relevant ARG.

Antimicrobials: CARBA – carbapenems; 3rd-GCs – third-generation cephalosporins; COL – colistin; FQ – fluoroquinolone; LIN – linezolid; Other acronyms: AMU – antimicrobial usage; GGP – Great Grandparent flock; CRE – carbapenem-resistant Enterobacterales; ND – not determined.

3.2.3.3. Cattle production sector

Examples of ARB and ARG found in the different potential AMR environmental sources identified in the cattle production sector are presented in Table 9. More detailed information can be found in Appendix F.2.2. Supporting references are included in the tables and appendix text.

In general, there is a shortage of studies focusing on AMR spread from environmental sources. This is especially the case for post-harvest.

A variety of bacteria carrying AMR can be found in cattle. In addition to studies focused on reporting resistance in *E. coli* and *Salmonella enterica* and monitoring existing and emerging resistances such as ESBL and colistin resistance, MRSA has also been investigated.

MRSA, including lineages associated with human infection (e.g. ST398- and CC97-carrying *mecA,* and the *mecC*-containing lineages CC130 and ST425), have been described in environmental sources, with wildlife species such as hedgehogs reported to be an important reservoir (Rasmussen et al., 2019).

Very little data is available relating to AMR *Campylobacter,* but some strains carrying relevant resistance mechanisms, have previously been linked to water sources.

E. coli, including lineages associated with human infection, and other Enterobacterales carrying genes encoding for ESBL or AmpC-related extended spectrum cephalosporin resistance are widely distributed in cattle production and have been found in diverse environmental sources including pasture soil, surface water, farm animal housing and equipment and flies.

Resistance to critically important antimicrobials, such as colistin resistance encoded by *mcr*-genes, has also been found in isolates obtained from environmental sources related to cattle, often linked to other resistance profiles such as ESBL on MDR plasmids. Moreover, transferable genes conferring resistance to carbapenems were found in *Acinetobacter* sp. in cattle-related samples and is likely linked to environmental sources.

Table 9: Distribution of antimicrobial-resistant bacteria and resistance genes in potential environmental sources of cattle (dairy/beef) farms: examples based on European literature

Main sources	Bacteria [antimicrobial resistance profiles and genes]$^{(a)}$	Detection of Group 1 ARB/highest relevant ARG$^{(d),(e)}$/comments	Factors influencing persistence and occurrence	Supporting references$^{(f)}$
Manure/Litter	*Salmonella* Dublin [MDR]	**–/–** IncA/C plasmids carrying $bla_{\text{CMY-2}}$ mostly seen in the US	Those plasmids seem to have a high fitness cost, thus maintenance associated with a persistent selective pressure. Some isolates harbour hybrid virulence-resistance plasmids.	Fenske et al. (2019)
	Acinetobacter genomic species 15TU [CARBA; $bla_{\text{OXA-23}}$]	**?/+**	Mastitis antimicrobial treatment in the previous weeks to detection.	Poirel et al. (2012)
	E. coli [3rd-GCs; $bla_{\text{CTX-M-1}}$]	**?/+**		Hartmann et al. (2012)
	E. coli [3rd-GCs; $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-32}}$ and $bla_{\text{CMY-2}}$]$^{(b)}$	**?/+** Farms with high prevalence of positive samples at given point also had higher diversity of genes present in faecal samples.		Hordijk et al. (2019)
Workers/visitors	*E. coli* [3rd-GCs; $bla_{\text{CTX-M-1/61;}}$ $bla_{\text{CTX-M-15/28/88}}$]$^{(b)}$	**+/+** Different lineages (ST405 associated with human infections; ST3891 strain in a farm worker and cattle).	All positive farmers worked in farms that tested positive for ESBLs.	Dahms et al. (2015)
Run-off, faecal waste on pasture and farm slurry	[$bla_{\text{CTX-M-1}}$ cluster, $bla_{\text{CTX-M-9}}$ cluster, *mecA*]	**ND/+** ARGs detected in bacteriophages and DNA isolated from cattle faecal waste and slurry	Phages are vehicles for mobilisation of the environmental pool of ARGs that contribute to the maintenance and emergence of new resistances.	Colomer-Lluch et al. (2011)
	E. coli [3rd-GCs, SXT; $bla_{\text{CTX-M-1}}$]	**?/+**	Manure application (animal origin) or sewage (human origin) also possibility of dissemination with wastewater.	Hartmann et al. (2012)
Farm environment	*E. coli* [3rd-GCs; $bla_{\text{CTX-M14/17}}$, $bla_{\text{TEM-35 (IRT-4)}}$]	**?/+** ESBLs genes in IncK plasmids of isolates from cows, calves and environmental samples	Resistance associated with a highly promiscuous plasmid.	Liébana et al. (2006)
	E. coli [3rd-GCs; diverse $bla_{\text{CTX-M}}$, AmpC genes]	**?/+**	Risk factors linked to mastitis treatment and use of sealants as well as floor scrapers.	Gonggrijp et al. (2016)

Main sources	Bacteria [antimicrobial resistance profiles and genes]$^{(a)}$	Detection of Group 1 ARB/highest relevant ARG$^{(d),(e)}$/comments	Factors influencing persistence and occurrence	Supporting references$^{(f)}$
	E. coli [3rd-GCs; COL; *mcr-1* and *bla*$_{\text{CTX-M-1}}$]	**?/+** co-location of *mcr-1* and ESBL gene in a large HI2 plasmid.	High levels of extended spectrum cephalosporin use likely promoting the occurrence of plasmids carrying those genes.	Haenni et al. (2016)
Soil, pasture	*E. coli* [3rd-GCs, SXT; bla$_{\text{CTX-M-1}}$]	**?/+**	Co-resistance.	Hartmann et al. (2012)
Wildlife, rodents, arthropods	*Enterobacteriaceae* [CARBA; *bla*$_{\text{VIM-1}}$, *bla*$_{\text{KPC-2}}$, *bla*$_{\text{NDM-1}}$]	**?/+** Several sources		Köck et al. (2018)
	E. coli [MDR,*bla*$_{\text{TEM}}$, *floR*, *strA*, *sul2*, *tetA*]	**?/+** Flies with isolates overlapping resistance and PFGE profiles with cattle isolates		Rybaríková et al. (2010)
	MRSA [*mecC*]	**+/+** MRSA CC130 and CC1943 spread in hedgehogs of DK and Sweden. Wild rabbits also identified as carriers CC130 in Spain		Bengtsson-Palme (2017), Ruiz-Ripa et al. (2019), Rasmussen et al. (2019)
	Enterococcus faecium [VAN; *vanA*]	**?/+** Red-legged partridges		Silva et al. (2018)
Co-grazing/ companion animals	MRSA [*mecA*]	**?/+** Different STs including CC398	MRSA transmission between species and spill over from pig population.	Tavakol et al. (2012)
Others environmental sources specific for the sector: Waste milk	*E. coli* [3rd-GCs; diverse *bla*$_{\text{CTX-M}}$]	**?/+** ESBL producers in environmental samples, calves and cows and waste milk.	Cefquinome use (residues in waste milk)	Randall et al. (2014)

(a): Multidrug (MDR) and resistance phenotypes to last resort antibiotics in capital letters; acquired resistance genes italicised; the ones conferring resistance to last resort antibiotics are underlined.

(b): Diverse phenotypes and/or different resistance determinant combinations.

(c): Resistance genes detected by metagenomic or other approaches.

(d): Group 1 ARB (according to definition in Table 6) were present (+), absent (–) or no information provided to indicate their presence (?)/ARG of highest relevance (according to definition in Section 3.2.2) were present (+), absent (–) or no information provided to indicate their presence (?).

(e): Horizontally transferability of resistance is assumed for resistance phenotypes or genes usually acquired by this process (e.g. *bla*$_{\text{CTX-M}}$ and *mcr* genes).

(f): Source of the data presented in 'Bacteria [antimicrobial resistance profiles and genes]' column and in the categorisation as Group 1 ARB/highest relevant ARG.

Antimicrobials: CARBA – carbapenems; 3rd-GCs- third-generation cephalosporins; COL – colistin; SXT – trimthoprim/sulfamethoxazole; VAN – vancomycin; Other acronyms: ESBL: extended spectrum beta-lactamase; MRSA – meticillin-resistant *Staphylococcus aureus*; ST: multi-locus sequence type; ND – not determined.

3.2.3.4. Pig production sector

Examples of ARB and ARG found in the different potential AMR environmental sources identified in the pig production sector are presented in Table 10. More detailed information can be found in Appendix F.2.3. Supporting references are included in the tables and appendix text.

The presence of high priority bacteria and genes within the pig production chain environment has been demonstrated. Bacteria such as *Salmonella enterica, E. coli, Enterococcus* spp*.* and LA-MRSA are the main antimicrobial-resistant zoonotic pathogens and commensal organisms identified in published studies. The occurrence of these bacteria and genes in the environment (water, dust, drinkers, soil, wild animals, etc.) appears to be directly linked to their presence in pigs, resulting in a cycle of transmission of bacteria and ARGs between the animals and their environment.

S. Typhimurium and its monophasic variant *S.* 1,4,[5],12:i:-, frequently associated with MDR profiles, including to critically important antibiotics, are frequently isolated. Even metallo-β-lactamases genes such as *bla*$_{\text{VIM}}$ have been described in *Salmonella* isolated from farm environments.

MRSA is also frequently isolated in farm housing environments, for instance in dust and exhibits resistance to highly important antimicrobials. In addition, lineages such as ST398 which are linked to humans are reported on pig farms.

Intensive monitoring of *colistin*-resistant *E. coli* from pig farms reveals the widespread distribution of *mcr* genes in European pigs as well as resistance to CIAs other than colistin.

The presence of vancomycin-resistant enterococci is also reported in pig farm environments (e.g. manure, feeders and soil) with some isolates of lineages being associated with human infections.

Table 10: Distribution of antimicrobial-resistant bacteria and resistance genes in potential environmental sources of pig farm and processing facilities: examples based on European literature

Main sources	Bacteria [antimicrobial resistance profiles and genes][a]	Detection of group 1 ARB/highest relevant ARG[d],[e]/comments	Factors influencing persistence and occurrence	Supporting references[f]
Feed	*Salmonella enterica* [MDR; $\mathit{bla}_{\mathrm{TEM}}$, $\mathit{bla}_{\mathrm{PSE\text{-}1}}$, *aadA1*,*aadA2*, *aphA*, *cml*, *dfrA12*, *mef* (B), *sul3*]	**+/**–		Tassinari et al. (2019),
	Enterococcus faecium [MDR; *tet*(M), *tet*(L), *erm*(B)][b]	–**/**– Diverse ST, including lineages causing human infections (CC5)	Co-selection of ARGs by the use of Cu or Zn in the feed	Novais et al. (2013)
	Enterococcus faecalis [MDR; *aac(6′)-Ie-aph (2″)-Ia*, *tet*(M), *tet*(L), *erm*(B)][b]	–**/**–		Novais et al. (2013)
Water	*E. faecalis* [VAN; *vanA*, *tet*(M), *aac(6′)-Ie-aph (2″)-Ia*][b]	+/+ ST6, lineage associated with strains causing human infection	Dissemination of AMR, perpetuation between batches	Novais et al. (2013)
	E. faecalis [MDR; *tet*(M), *tet*(L), *erm*(B), *aac (6′)-Ie-aph(2″)-Ia*][b]	–**/**–		
	E. faecium [VAN; *vanA*, *aac6′-Ie-aph2″-Ia*]	**+/+** ST18, lineage associated with strains causing human infection.		
Air	*E. faecalis* [MDR; *aac(6′)-Ie-aph(2″)-Ia*, *tet*(M), *tet*(L)]	–**/+** ST21, lineage associated with strains causing human infection	Risk of transmission between rooms, facilities and to spread to other close farms	Novais et al. (2013)
	E. faecium [MDR; *tet*(M), *tet*(L)][b]	–**/**–		
Dust	*E. faecalis* [MDR; *tet*(M), *tet*(L), *erm*(B), *aac (6′)-Ie-aph(2″)-Ia*][b]	–/–	Risk of transmission between rooms, facilities and to spread to other close farms, risk of carriage by humans	Novais et al. (2013)
	E. faecium [MDR; *tet*(M), *tet*(L), *erm*(B)][b]	–/–		Novais et al. (2013)
	MRSA [*mecA*];	**+/+**		Pilote et al. (2019)
	[*mcr-1*][c]	**ND/+**		Pilote et al. (2019)

Main sources	Bacteria [antimicrobial resistance profiles and genes][(a)]	Detection of group 1 ARB/ highest relevant ARG[(d),(e)]/ comments	Factors influencing persistence and occurrence	Supporting references[(f)]
Equipment (feeders)	*E. faecium* [VAN; *vanA*, *aac(6′)-Ie-aph(2′′)-Ia*, *tet*(M), *tet*(L), *ermB*]	**+/–** CC5, associated with strains causing human infection	Source of feed contamination, spread of ARB	Novais et al. (2013)
Wild animals, arthropods	*S. enterica* (MDR; *tet*(G), *floR*, *dfrA12*, *aadA2*; *bla*$_{\text{PSE-1}}$)	–**/**–	Risk of contamination of animals and insects inside facilities after exposure to ARB	Caleja et al. (2011),
	S. Choleraesuis [AMP; *bla*$_{\text{TEM-1}}$]	–**/**–		Leekitcharoenphon et al. (2019)
	E. coli [CARBA; *bla*$_{\text{VIM-1}}$]	**?/+**		Fischer et al. (2017)
	E. coli [MDR, 3rd-GCs; *bla*$_{\text{TEM}}$, *strA*/*strB*, *aadA*, *sul1*, *sul2*, *tet*(A), *tet*(B), *tet*(C)] [(b)]	**?/+**		Guenther et al. (2010) Himsworth et al. (2016)
Farm surroundings	*Salmonella* Infantis [CARBA; *bla*$_{\text{VIM-1}}$]	**+/+**	Risk of spread from farm to other environments or to re-introduction of ARB into the farm	Fischer et al. (2013)
	E. coli [CARBA; *bla*$_{\text{VIM-1}}$]	**?/+**		Fischer et al. (2017)
Soil (extensive production)	*Enterococcus* spp., [VAN; *vanA*, *tet*(M), *tet*(L), *erm*(B)]	**+/+** CC5, associated with strains causing human infection	Risk of spread and perpetuation of AMR on the farm, risk of spread by fomites to other environments.	Novais et al. (2017)
	[*cfr, optrA*][(c)]	ND/– Linked to bacteria from families *Peptostreptococcaceae* and *Streptococcaceae* by shotgun seq analyses	Risk of perpetuation of AMR on farms, risk of HGT between commensals and pathogens	Mencía-Ares et al. (2020)
Post-harvest				
Workers	[*tet*(W), *erm*(B)][(c)]	**ND/**–	Risk of introduction and re-circulation of AMR in the food chain	Van Gompel et al. (2020)

(a): Multidrug (MDR) and resistance phenotypes to last resort antibiotics in capital letters; acquired resistance genes italicised; genes conferring resistance to last resort antibiotics are underlined.
(b): Diverse phenotypes and/or different resistance determinant combinations.
(c): Resistance genes detected by metagenomic or other approaches.
(d): Group 1 ARB (according to definition in Table 6) were present (+), absent (–) or no information provided to indicate their presence (?)/ARG of highest relevance (according to definition in Section 3.2.2) were present (+), absent (–) or no information provided to indicate their presence (?).
(e): Horizontally transferability of resistance is assumed for resistance phenotypes or genes usually acquired by this process (e.g. *bla*$_{\text{CTX-M}}$ and *mcr* genes).
(f): Source of the data presented in 'Bacteria [antimicrobial resistance profiles and genes]' column and in the categorisation as Group 1 ARB/highest relevant ARG.
Antimicrobials: CARBA – carbapenems; VAN – vancomycin; Other acronyms: MRSA – meticillin-resistant *Staphylococcus aureus*; ST: multi-locus sequence type; ND – not determined.

3.2.3.5. Aquaculture production sector

Examples of ARB and ARG found in the different potential AMR environmental sources identified in the aquaculture sector are presented in Table 11. More detailed information can be found in Appendix F.3. Supporting references are included in the tables and appendix text.

Few data exist for AMR priority pathogens in aquaculture production systems due to a lack of routine surveillance and a focus on indicator species and indigenous aquatic fish pathogens.

Transferable resistance to highly important antimicrobials was described in *E. coli* strains harbouring resistance genes to fluoroquinolones or extended-spectrum cephalosporins, from water and sediments from a trout farm. *K. pneumoniae* harbouring fluoroquinolone resistance genes (*qnrB7, oqxA* and *oqxB*) and *Salmonella* serovars with colistin resistance were also reported from trout farms.

Information collected from studies in bivalve molluscs also documents contamination with MDR *Salmonella* serovars, including an isolate bearing *mcr-1* conferring colistin resistance and of ESBL *E. coli* from farmed and wild shellfish in Europe (carbapenemase-producing *E. coli* isolated from venus mussels gathered at retail have been described, Roschanski et al., 2017a).

Moreover, indigenous fish pathogens include opportunistic human pathogens such as *Aeromonas* spp., some of which possess chromosomal (non-mobile) carbapenem resistance genes and have been suggested to be the origin of transferable colistin resistance genes, e.g. *mcr-3* (Table 11, Appendix F, Table F.2).

Table 11: Distribution of antimicrobial-resistant bacteria and resistance genes in potential environmental sources of contamination of aquaculture and processing facilities: examples based on European literature

Main sources (risk factors)	Bacteria [antimicrobial resistance profiles and genes](a)	Detection of highest relevant ARB pathogens/ ARG(d),(e)/comments	Factors influencing persistence and occurrence	Supporting references(f)
Primary production			In some cases the source of specific organisms in water and sediment can be attributed to either human faecal waste (where isolates are known to be human adapted or ARGs are associated with clinical isolates) or from livestock waste (where strains are animal adapted sequence types or genes are predominantly associated with animal strains). Other species such as *Aeromonas* spp. and *Vibrio* spp. are indigenous environmental organisms. Microbial source tracking methodologies are used, and are being further developed, to identify aquatic microbial pollution sources.	
Freshwater	*S. Abony* [COL, STR]]	**+/?**	Low FQ and TET concentrations in water of aquaculture facility.	Antunes et al. (2018)
	S. Newport [STR, KAN]	**?/?** ST118		
	E. coli [FQ; *qnrS1*; *bla*$_{\text{TEM}}$]	**?/+**		
	E. coli [3rd-GCs; *bla*$_{\text{SHV-12}}$; *strA/strB, tet*(B)]	**?/+**		
	E. coli [AMX, AZT, CAZ, 3rd-GCs, TET, *bla*$_{\text{SHV-12}}$, *str*A-*str*B, *tet*(B)]	**?/+** Samples taken upstream, in trout farm or downstream.		
	E. faecalis [MDR; *tet*(M), *tet*(L), *erm*(B), *aadE, cat_pC221*](b)	**?/?**	Low FQ and TET concentrations in water of aquaculture facility	Novais et al. (2018)
	E. faecium [MDR; *tet*(M), *tet*(L), *erm*(B), *aadE*](b)	**?/?**		
Seawater	*E. casseliflavus*; [TET, AMP; *tet*(L), *tet*(M), *blaZ*]	**?/?**		Di Cesare et al. (2012)
	E. durans [AMP; *blaZ*]	**?/?**		
	E. faecalis [AMP, TET; *tet*(M), *tet*(K), *blaZ*]	**?/?**		
	E. faecium [MDR; *tet*(M), *tet*(K), *blaZ*)]	**?/?**		

Main sources (risk factors)	Bacteria [antimicrobial resistance profiles and genes](a)	Detection of highest relevant ARB pathogens/ ARG(d),(e)/comments	Factors influencing persistence and occurrence	Supporting references(f)
	Enterococcus spp. [AMP, TET; *tet*(L), *tet*(K), *blaZ*]	**?/?** Mediterranean aquaculture sites		
Freshwater sediment	*E. coli* [AMX, FQ; KAN, STR, TET/*bla*$_{TEM}$, *qnrS3, aphA1, aadA, tet*(B)] *Citrobacter gillenii* [KAN, STR, TET; *qnrS3, strA/strB*] *Klebsiella pneumoniae* [CHL, FQ, STR, SUL, TET, TMP/*catA, aadA, sul*1, *dfrA*1, *qnrB7, oqxA, oqxB*]	**?/?** **?/+** **+/+** Trout farm		Antunes et al. (2018)
Marine sediment	*E. casseliflavus*; [AMP; *blaZ*] *E. faecalis* [AMP; *blaZ*] *E. faecium* [MDR; *tet*(M), *tet*(L)] *E. gallinarum* [TET; *tet*(M)] *Enterococcus* spp. [AMP, TET; *tet*(M), *tet*(L), *blaZ*]	**?/?** **?/?** **?/?** **?/?** **?/?** Mediterranean aquaculture sites		Di Cesare et al. (2012)
Feed	*E. hirae* [MDR; *tet*(M), *tet*(L), *erm*(B)]	**?/?** *tcrB, cueO* present		Novais et al. (2018)
	K. pneumoniae [FQ; *oqxAB; cmlA, aphA1, aadA, sul3, tet*(B), *dfrA12*]	**+/+**		Antunes et al. (2018)
Fish and bivalve molluscs(g)				
Bivalve molluscs	*Salmonella* serovars [MDR] *Salmonella* Rissen *aac(6′)-Iaa, aadA1, aadA*2, *bla*$_{TEM-1B}$, *cmlA1, sul1, sul3, tet*(A), *dfrA1* and *mcr*-1	**+/+** **+/+** farmed mussels		Lozano-León et al. (2019)
	E. coli [3rd-GCs; *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-14}$]	**?/+** farmed and wild mussels		Grevskott et al. (2017)

Main sources (risk factors)	Bacteria [antimicrobial resistance profiles and genes][(a)]	Detection of highest relevant ARB pathogens/ARG[(d),(e)]/comments	Factors influencing persistence and occurrence	Supporting references[(f)]
Fish in freshwater	*Aeromonas hydrophila* complex and *A. veronii* [*cph*A]	**?/–** *cph*A is an intrinsic ARG conferring resistance to carbapenems when induced by carbapenem exposure. *Aeromonas* spp. are fish and opportunistic human pathogens		Smyrli et al. (2019)

*: MIC below clinical breakpoints.

(a): Multidrug (MDR) and resistance phenotypes in capital letters; acquired resistance genes italicised; genes conferring resistance to last resort antibiotics are underlined.

(b): Diverse phenotypes and/or different resistance determinant combinations.

(c): Group 1 ARB (according to definition in Table 6) were present (+), absent (–) or no information provided to indicate their presence (?)/ARG of highest relevance (according to definition in Section 3.2.2) were present (+), absent (–) or no information provided to indicate their presence (?).

(d): Horizontal transferability of resistance is assumed for resistance phenotypes or genes usually acquired by this process (e.g. *bla*$_{\text{CTX-M}}$ and *mcr* genes).

(e): Source of the data presented in 'Bacteria [antimicrobial resistance profiles and genes]' column and in the categorisation as Group 1 ARB/highest relevant ARG.

(f): Fish and bivalve molluscs contamination by relevant ARB are indicators of AMR contaminated sources at preharvest or post-harvest processes.

Antimicrobials: AMX – amoxicillin; AMP – ampicillin; AZT – aztreonam; CARBA – carbapenem; CHL – chloramphenicol; COL – colistin; 3rd-GCs – third-generation cephalosporins; FQ – fluoroquinolone; KAN – kanamycin; STR – streptomycin; SUL – sulfonamide; TET – tetracyclines; TMP – Trimethoprim. Other acronyms: ST: multi-locus sequence type; ND – not determined.

3.2.4. Factors for occurrence and persistence of antimicrobial-resistant bacteria and resistance genes in food-producing environments

As indicated in the introduction of this opinion and in Section 3.1, there are several general factors that contribute to the occurrence and persistence of ARB and ARG in food-producing environments. Specific factors inherent to those bacteria (e.g. genetic content, MGE, virulence, co-localisation of resistance genes, stress response and biofilm formation capability) may also be involved. Some examples of both categories are described below and included in Tables 7–11. More information is provided in Appendices E and F.

AMU, either recently or historically, within the food animal breeding and production sectors is the main factor that has selected for ARB at farm level. AMU has been decreasing in recent years in most food animal sectors, especially aquaculture and poultry, in most EU MSs (EMA, 2020), although intermittent therapeutic or environmental exposure to antimicrobials and co-selection, including by heavy metals used in animal feed, are still relevant factors. Co-selection is particularly relevant for resistances encoded by genes, e.g. ESBL or *mcr-*, located on the same MDR plasmid as genes for more commonly used antimicrobials (Gazal et al., 2021).

Once introduced, ARB can circulate as a result of inadequate definition or implementation of biosecurity measures and food safety management systems (FMMS) with ineffective food hygiene procedures, e.g. GMP/GHP/PRP/HACCP. The cycle of contamination and re-contamination of animals and their environment, with involving faecal matter and skin contamination/colonisation, is considered to be the main contributor to persistence of ARBs in the farm environment and individual or groups of animals. Moreover, the organisms found to harbour resistance will also circulate between the animals and different sources within the farm environment. Robust organisms such as *S. enterica*, *E. coli*, *Klebsiella* spp., MRSA and *Enterococcus* spp. can persist in the farm, hatchery or slaughter/processing environment as a result of inadequate cleaning and disinfection, biocide tolerance, especially to quaternary ammonium compounds (QACs) (Al-Johny and Alkhuzaee, 2019; Aksoy et al., 2020; Pang et al., 2020) recontamination from the external environment or reintroduction by movement of people, replacement animals, wildlife vectors, bioaerosols or contaminated equipment (Persoons et al., 2010; Swaggerty et al., 2018; Voss-Rech et al., 2019; Castañeda-Gulla et al., 2020). Exposure to certain biocides/heavy metals can also select for AMR and increase genetic transfer between bacteria (Silveira et al., 2013; Dunn et al., 2020). Less robust organisms, such as *Campylobacter* spp., can persist within animal housing for sufficient time to infect subsequent flocks/herds if cleaning and disinfection is poor, but are more likely to persist in the external farm environment, and the same strain can be transferred within and between farms by the movement of personnel and equipment (EFSA BIOHAZ Panel, 2020).

Persistence of resistance, such as to glycopeptides in populations of bacteria such as *E. faecium* or to extended spectrum cephalosporins in *E. coli,* may also involve transfer of resistance genes between diverse commensal, environmental and pathogenic bacterial species and clones occurring within the farm environment (Sørum et al., 2006; Schwaiger et al., 2013), whereas high level resistance to fluoroquinolones in bacteria such as *Campylobacter* and *Salmonella* is predominantly chromosomally mediated and persistence is a result of the failure to eliminate specific resistant clones (Monte et al., 2019; Perrin-Guyomard et al., 2020). It is often assumed that there will be a fitness cost to maintenance of ARGs that will reduce persistence of ARB, but this is not the case where there are compensatory mechanisms and the fitness cost can vary in different environments (Clarke et al., 2020). In the case of fluoroquinolones, *Campylobacter* clones with resistance may show enhanced environmental resilience and virulence (Whelan et al., 2019).

Biofilm formation and the degree of relevant gene expression may also be important for persistence of *Salmonella* within the host and in the environment, including for certain strains of MDR *S*. Typhimurium and *S*. Heidelberg (Hoffmann et al., 2014; Shi et al., 2019). Biofilms also reduce the effectiveness of antimicrobials, which may lead to greater selection of resistance (Penesyan et al., 2020) and reduce the activity of disinfectants (Nesse et al., 2021).

Salmonella Heidelberg is a serotype that is globally associated with invasive MDR infections, including outbreaks linked to poultry meat and eggs (Souza et al., 2020) and can also carry phages and plasmids with diverse virulence factors (e.g. P2-like phage-*sopE1* gene, IncX-T4SS) which could play a role in their virulence, colonisation ability and persistence. A recent example of MDR in *S.* Infantis is also of interest because of the involvement of a promiscuous pESI-like megaplasmid (Alba et al., 2020; Szmolka et al., 2021) that encompasses AMR and virulence/fitness traits (including toxin-antitoxin systems) that are thought to have been involved in the rapid international dissemination of various clones in the broiler industry via environmental routes, despite limited involvement of the breeding sector, and possibly also selected by the regular use of preventive antimicrobials for chicks prior to recent prudent use initiatives (EFSA, 2019; Alba et al., 2020).

MRSA ST398, is a robust organism that can resist desiccation, harsh environments and standard disinfection programmes and is therefore able to persist in the farm environment for a long period and infect pig herds, poultry flocks and the mammary glands of dairy cows (Ribeiro et al., 2018; Barberio et al., 2019; Rodríguez-López et al., 2020).

In plant-based food production, although most bacterial contamination is on the surface of the plant, and originates from the production environment (including inputs such as manure and contaminated water), the processing environment or food handlers, there is also a concern that some pathogenic bacteria (e.g. *Salmonella*) can become internalised in plant tissues during growth of the plant (Burris et al., 2020), making such contaminants impossible to remove from raw products through processing or kitchen preparation. The persistence and uptake into crops (e.g. lettuce, corn salad) of bacteria varies with species, soil type and crop (Teplitski and de Moraes, 2018). Antimicrobials in the horticultural environment can also become internalised (El Gemayel and Bashour et al., 2020).

There is, to our knowledge, no European literature systematically evaluating factors determining the risk of AMR contamination in the post-harvest processing environment of food animal products or produce, with most studies being focused on single organisms or a limited number of study situations (Bennani et al., 2020).

There is little published evidence relating to long-term persistence of ARB in food animal slaughter environments. Several different clones of different MDR *Salmonella* serovars isolated by placing polystyrene mats beneath a poultry processing line were able to persist on surfaces for several weeks and showed substantial biofilm formation (Dantas et al., 2020), which supports longitudinal studies that have identified resident MDR *Salmonella* in the poultry slaughterhouse environment (Shang et al., 2019), as well as ineffective cleaning and disinfection of poultry transport crates (Moazzami et al., 2020). Persistent AMR and heavy metal resistant bacteria that have enhanced stress response characteristics and biochemical changes that compromise detection may be selected after exposure to biocides (Mourão et al., 2020; Rhouma et al., 2020). *L. monocytogenes* strains isolated from the poultry processing environment can also form biofilm, with benzalkonium chloride-based cleaning programmes showing limited efficacy and exacerbating biofilm formation and AMR expression (Cadena et al., 2019; Rodríguez-Campos et al., 2019; Puangseree et al., 2021).

3.2.5. Concluding remarks on antimicrobial-resistant bacteria and resistance genes of highest public health relevance in food-producing environments, and risk factors for occurrence and persistence

- In food-producing environments, resistance to antimicrobials of choice for the treatment of serious bacterial infections or to last resort antibiotics was identified in bacterial pathogens (highest priority Group 1 bacteria) and in commensals or environmental bacteria encoded by mobile genetic elements (highest priority Group 2 bacteria). Those ARGs usually associated with mobile genetic elements are considered of highest priority.
- Resistance to extended spectrum cephalosporins and fluoroquinolones was commonly reported. Carbapenems, colistin and glycopeptides resistance was also identified in bacteria/genes from different sources within plant-food and terrestrial animal food-producing environments. Resistance to oxazolidinones and plazomicin was rarely identified. For the aquaculture sector, reports of resistance to these antimicrobials were scarce or absent.
    - Among the highest priority Group 1 bacteria, extended-spectrum cephalosporin/ fluoroquinolone resistant MDR Enterobacterales (including *Salmonella enterica*) were identified in several sources and sectors. Fluoroquinolone- resistant *Campylobacter* spp. was particularly identified in poultry and plant-producing environments, MRSA in cattle, pigs and plant productions and VRE in pig and plants production. Carbapenem or colistin resistance in Gram negative pathogens was more rarely reported.
    - Highest priority Group 2 bacteria were frequently identified in several sources and sectors. MDR Enterobacterales (mostly *E. coli* and *K. pneumoniae*) resistant to extended spectrum cephalosporins and/or fluoroquinolones were common (e.g. in manure from various animal species). Colistin and carbapenem transferable ARGs were also described in different bacterial species (e. g. *bla*$_{\text{OXA-23}}$ in *Acinetobacter* spp.) from different environmental contexts (e.g. carbapenem resistant *E. coli* in wildlife and environment of piggeries). Extended-spectrum cephalosporin and colistin mobile resistance genes were simultaneously identified

  in *E. coli* isolates. Glycopeptide resistance in *E. faecium* or *E. faecalis*, as well as oxazolidinones resistant enterococci were also identified.
  - Among the highest priority ARGs, those conferring resistance to carbapenems (e.g. *bla*$_{\mathrm{VIM}}$; *bla*$_{\mathrm{NDM}}$, *bla*$_{\mathrm{OXA\text{-}48\text{-}like}}$, *bla*$_{\mathrm{OXA\text{-}23\text{-}like}}$), extended-spectrum cephalosporins (e.g. *bla*$_{\mathrm{CTX\text{-}M}}$, *bla*$_{AmpC}$), plazomicin (*armA*), colistin (*mcr* genes), beta-lactams (*mecA, mecC*), glycopeptides (*vanA*) and oxazolidinones (*cfr*, *optrA*) have been reported from food production environments. The few descriptions so far of some of these genes (e.g. rRNA methylases and oxazolidinones resistance genes) may reflect the limitations in the testing strategies focused on few bacteria and antimicrobial resistance profiles or ARGs.

- Highest priority ARB and ARGs identified in the food production environments could originate from several sources, including manure, water, workers and wildlife at primary production and transport, lairage, slaughtering and meat processing at post-harvest level.
- Several general factors facilitating the occurrence of ARB and ARG were identified, including selective pressure by different compounds (e.g. use of antimicrobials, heavy metals or biocides), introduction of ARB and ARG via breeding pyramids, continuous cycling of bacteria between the animals and their environment, resulting from inadequate definition or implementation of biosecurity measures and food safety management systems with ineffective food hygiene procedures, e.g. GMP/GHP/PRP/HACCP.
- Factors linked to the general resilience (e.g. resistance to desiccation; temperature) and biofilm formation of specific ARB strains are relevant to their persistence in the food production environment. Moreover, transferability of ARGs, location of ARGs conferring resistance to different antimicrobials on the same genetic platform (e.g. chromosome, plasmid or genomic island encoding a gene conferring another resistance determinant), or on the same MGE as genes conferring metal and/or biocide tolerance and involved in stress response, biofilm formation and virulence, as well as compensatory mechanisms to minimise the fitness cost of replicating ARGs, are all relevant to the successful extension and persistence of ARB.
- Replacement of animals and persistent environmental contamination are important factors involved in recontamination.
- The microbiota of natural environments, particularly soil, slow-moving water or sediment, is a natural reservoir and source of ARGs that could contribute to the occurrence and persistence of ARB/ARG in the food-producing environment.

3.3. Strategies and options to mitigate the emergence and spread of antimicrobial-resistant bacteria and resistance genes in food-producing environments

This section will describe mitigation measures and strategies that are currently in use for preventing and/or reducing the spread of AMR in the food-producing environment, as well as other measures which could be implemented in the future. These measures are intended to complement AMU stewardship initiatives in animal production and human medicine, which are expected to have the greatest impact on the occurrence and levels of transferable AMR in food production and in waste streams potentially contaminating the food production and wider environment. Some of these measures are general for all the production systems included in this opinion, whereas others may be specific for a particular sector or subsector.

In general, current food safety practices throughout the farm to fork continuum are designed to reduce the risk of contamination by pathogenic bacteria, and not specifically to reduce the risk of AMR selection/transmission, whether by pathogenic or non-pathogenic bacteria. Only a few measures specifically target the emergence and spread of ARB and ARGs. Most of these focus on ARB, but may also impact on ARGs, MGEs and antimicrobials, although there is less evidence on these aspects, particularly in an EU context. General measures aimed at prevention and control of AMR-related risk pathways are summarised below. Information is also provided on mitigation measures applied in specific production sectors considered in the previous sections (plant-based foods, terrestrial animals and aquaculture). In some cases, the sources, and thus, measures applied are common to the different sectors (e.g. manure, feed, etc.). Mitigation measures to avoid/reduce contamination through water have been addressed in Section 3.3.1.4. Potential measures that could be implemented in the future are also described in Section 3.3.2.

General mitigation measures

In general, AMU contributes to selection for pre-existing ARB and spontaneous ARB mutants, emergence of novel ARGs in pathogens originating in commensals and environmental bacteria, increased horizontal transfer of MGEs/ARGs in some cases, an increase in the shedding of ARB and the release of antimicrobial residues into the environment. All measures aiming to reduce AMU and other co-selective agents such as heavy metals would also be expected to reduce the occurrence of ARB, ARGs and antimicrobial residues in the food-producing environment. The relevant measures to reduce the use, and the need to use antimicrobials (e.g. biosecurity, good hygiene and implementation of practices that promote health and prevent disease outbreaks) have been extensively reviewed in recent reports such as the RONAFA scientific opinion (see Appendix B) prepared by EMA and EFSA (EMA and EFSA, 2017). The Veterinary Medicines Regulation (Regulation (EU) 2019/6)[36] will introduce a 'toolbox' of measures and actions to promote the prudent use of antimicrobials including rules for the mandatory collection of data on sales and use, reserving antimicrobials for human use; antimicrobials that cannot be used under the cascade or subject to certain conditions, restricting prophylaxis and metaphylaxis use as well as promoting innovation that can lead to development of new and alternatives to antimicrobials thus contributing to strengthening the EU action against AMR. For this reason, we will refer to these reports/legislation and will not consider mitigation measures related to AMU, use of disinfectants, heavy metals or other biocides with potential for co-selection or promotion of gene transfer in this opinion. Likewise, the release of antimicrobial residues into the environment, and measures aimed at the reduction or elimination of these residues, is not within the scope of the present opinion.

Also important are general management strategies that focus on prevention and control of the spread of ARB originating from primary food animal production and, to a lesser extent, from contamination in the course of harvesting, processing and distribution. Such measures refer to the effective application of good hygienic practices (mainly covered by Regulation (EC) No 852/2004[37] and Regulation No 853/2004[38]) throughout the food chain, which provides a varying degree of assurance against the introduction of certain ARB onto or into food, as has been demonstrated in the case of known pathogens and the control of spoilage bacteria (Abdel-Aziz et al., 2016; Panghal et al., 2018).

3.3.1. Mitigation measures

3.3.1.1. Measures for mitigation of AMR in the plant-based food production sector

As recommended by EFSA BIOHAZ Panel (2014a–e), FAO/WHO (2019a) and Codex Alimentarius (FAO, 2003; FAO/WHO, 2019b), good hygiene practice is designed to reduce the risk of food exposure to pathogenic microorganisms, regardless of whether they are AMR or not. A fundamental question is the efficacy and optimal implementation of these guidance documents and codes for hygienic practice with respect to preventing contamination of plant-based foods by environmental AMR. Gil et al. (2015) published an overview of the most important preventive measures along the farm to fork chain to prevent microbial contamination of leafy greens, including technological and managerial interventions related to primary production, post-harvest handling, processing practices, distribution and consumer handling to eliminate pathogens.

For this sector, the most important sources identified in Section 3.1.1 were contamination by human and animal associated ARB found in manures, soil and faecally contaminated water used for growing, irrigating and processing crops. Although aquatic and soil environments constitute a reservoir of AMR even in the absence of contamination, high prevalence of clinically important ARB and ARGs is associated with contamination.

Soil

Soil, or the growth matrix in the case of soil-free cultivation (Kasozi et al., 2021), has particular importance in the plant-based food sector because of the direct contact of plants with the soil/growth matrix and its microbiota, which can be a source of ARB and ARGs. Actions to reduce the spread of

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[36] Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC. OJ L 4, 7.1.2019, p. 43–167.

[37] Original regulation and amendments shown in the consolidated text of 2009/04/2020, https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02004R0852-20090420&from=EN

[38] Original regulation and amendments shown in the consolidated text of 2009/04/2020, https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02004R0853-20210101&from=EN

ARB include avoiding irrigation practices that physically disturb the soil and cause splashing, as well as ensuring good hygiene practices to remove soil from food products, equipment, holding bins, etc. during harvest, transportation and processing is relevant (Mogren et al., 2018).

Preventive measures to avoid the presence of pathogens and enrichment of soils with ARB and/or ARGs through application of manure, irrigation with faecally-contaminated water (e.g. reclaimed water or surface water containing wastewater treatment effluent or animal faeces) are important. Prior grazing of land by livestock or contamination by wildlife or other domestic species are also relevant considerations in some horticultural settings (Alegbeleye et al., 2018). Ensuring there are no livestock in adjacent fields is also important (Ridley et al., 2011).

Soil amendment application techniques must control, reduce or eliminate the likely contamination of surface water and/or edible crops being grown (FAO/WHO, 2008; WGA, 2018). Efficient preventive measures to minimise risk include the establishment of suitable conservative preplant or preharvest intervals, which should be appropriate for specific crops and regional and field conditions (Suslow et al., 2003; Gil et al., 2015).

There is a risk that some ARB may be translocated from manured soil into plant tissues (Jo and Park, 2019). Once internalised, bacteria will be protected from post-harvest sanitary measures. The probability of this happening can be reduced by treating manure (by effective composting or anaerobic digestion measures) prior to application, which would reduce the high concentration of bacteria that would pose a much greater risk of internalisation (Hirneisen et al., 2012). Implementing a suitable delay between application of non-or incompletely treated (e.g. by aerobic digestion without prior heat treatment) manure and the germination, growth or harvest of crops intended for human consumption will also reduce internalisation, as well as surface contamination (Sharma and Reynnells, 2018; Ekman et al., 2020).

In addition, climate is likely to be an important factor in relation to environmental contamination and may become increasingly so with ongoing changes (MacFadden et al., 2018). Higher temperatures may be associated with increased survival or persistence of human and animal associated bacteria in the environment, and high rainfall events can disseminate bacteria from farms and sewage systems to river catchments. These climatic factors should be considered when planning future mitigation methodologies and capacity (Demeter et al., 2021).

Measures to reduce contamination of plant-based foods by AMR carried in manures

Animal faeces represents a source of ARB into crop production systems directly through application of manure or by animals excreting on pasture, and indirectly through usage of surface water affected by run-off from manured fields or by municipal wastewater. In many confined livestock and poultry production systems, the manure is stored (e.g. in pits, tanks, lagoons or mixed with bedding) for a period of time, to be later used as fertiliser in the form of slurry or compost applied directly to the land. Furthermore, the farmer may further store, compost or treat the manure prior to land application (Ruiz-Barrera et al., 2020). In this regard, best available techniques for use of manure on land and techniques for on-farm manure processing are included in Commission implementing Decision 2017/302[39]. In production systems where animals or poultry are on pasture, their excreta may be further disseminated via run-off, wind-borne dust or wildlife. Modification of cattle diets, to reduce pathogen concentrations in manure, has been reported as a preventive measure, although results among studies vary considerably, thus they would be controversial for formation of control policies (Gil et al., 2015).

Manure stored prior to land application can be treated in order to reduce the microbial load and consequently ARBs. This may involve different types of treatment (Youngquist et al., 2016), such as those described below:

- Composting is the microbial process by which organic matter is decomposed and nutrients such as nitrogen are stabilised under aerobic conditions. The process is exergonic, and the heat produced contributes to reducing the abundance of enteric bacteria and many of the ARB and ARGs they carry. This consequently reduces the risk of contamination of plant-based foods that are produced in or close to manured soil, such as vegetables (Guron et al., 2019; Subirats et al., 2020). Composting can be employed by farms that handle manure as a slurry, if separation of solids is also implemented. Optimisation of the composting process may be problematic, particularly with respect to maintaining optimal aeration and moisture content but the addition

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[39] Commission Implementing Decision (EU) 2017/302 of 15 February 2017 establishing best available techniques (BAT) conclusions, under Directive 2010/75/EU of the European Parliament and of the Council, for the intensive rearing of poultry or pigs (notified under document C(2017) 688). OJ L 43, 21.2.2017, p. 231–279.

  of plant-based biochar or use of vermiculture can assist the bacterial degradation process (Liu et al., 2020; Ngigi et al., 2020).
  - Anaerobic digestion is the microbial process by which, under anoxic conditions, organic matter is decomposed and reduced, with the formation of methane and hydrogen, which can be used to produce energy. Anaerobic digestion systems are generally employed because of the valuable biogas that they produce, the value of the digestate as fertiliser, and the reduction of sludge volume which reduces transportation costs. Anaerobic digestion will inactivate some pathogenic organisms and reduce the abundance of some ARGs (Tien et al., 2017; Jiang et al., 2020), but increased transfer of MGEs can occur under some circumstances (Zhang et al., 2020a,b). Anaerobic digestion equipment requires expertise to operate. Thermophilic systems are much more effective for ARB and ARG reduction than mesophilic processes (Youngquist et al., 2016). Generally, they operate with slurry feedstocks, rarely with solids (Tien et al., 2017; Jiang et al., 2020). Enhanced methodologies can substantially increase the removal of ARB/ARGs, as well as some chemical contaminants such as antimicrobials, and merit further investment (Congilosi and Aga, 2020; Han et al., 2020).
  - Coupled nitrification–denitrification is a sequential microbial process by which, under aerobic conditions, manure ammonium compounds are oxidised to nitrates, and then under anaerobic conditions the nitrate is reduced to dinitrogen gas that is then lost to the atmosphere. The process facilitates processing of excess manure in relation to the local land capacity and can reduce the bacterial load by up to 2 logs. A recent study (Van den Meersche et al., 2019) showed a concurrent reduction of the level of zoonotic bacteria and certain ARGs in the end-product after biological nitrogen removal from pig manure. However, this methodology is not applicable for new facilities according to Commission implementing Decision 2017/302.

It is important to note that none of these manure treatment practices were designed with the purpose of eliminating ARB or ARGs, and current available evidence indicates that not all ARB and ARGs will dissipate during the process. Additional research would be of value to determine optimum anaerobic digestion and composting conditions for removal of ARB and to increase understanding of the fate of ARGs during anaerobic digestion and composting (Youngquist et al., 2016). In general, improved treatment of faecal waste will reduce transmission of faecal pathogens but increases storage and equipment resources requirements and may reduce the fertiliser value.

- Delays between manure application and harvest. There are several publications on increases in AMR in agricultural soils after manure fertilisation. It is not entirely clear if weeks or months are required to reduce ARB and ARG abundance to background levels following a manure application, and this is likely to vary according to the type and abundance of ARB, the type of soil and the local climatic conditions (Marti et al., 2014; Pu et al., 2019). Examples of good practices for preharvest intervals that should be followed when growers use organic fertilisers can be found in the Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene,[40] e.g. for land used to grow fresh fruit and vegetables eaten raw, treated manures can be applied at any time before drilling/planting, but raw manure should not be applied within 12 months of harvest and at least 6 months before drilling/planting.

Measures to reduce bacterial contamination through sewage sludge

Contamination of plant-based foods with microbial or chemical contaminants carried in sewage sludge is a particular human and animal health concern. In this context, the EU mandates a minimum ten-month delay between the land application of sewage sludge and the harvest of produce grown in that ground (Rizzo et al., 2020). In fact, the Sewage Sludge Directive (Directive 86/278/EEC)[41] specifies that to provide protection against potential health risks from residual pathogens, sewage sludge must not be applied to soil in which fruit and vegetable crops are growing or grown (with the exception of fruit trees), or within at least ten months before harvest of fruit and vegetable crops, which are normally in direct contact with the soil and normally eaten raw. Furthermore, the use of untreated sludge on agricultural land is prohibited, unless it is injected or worked into the soil (MSs can authorise this use under conditions laid down by them). Treated sludge is defined as having

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[40] OJ C 163, 23.5.2017, p. 1–40.

[41] Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. OJ L 181, 4.7.1986, p. 6–12.

undergone 'biological, chemical or heat treatment, long-term storage or any other appropriate process so as significantly to reduce its fermentability and the health hazards resulting from its use'. Depending on the type of treatment (conventional vs. enhanced), the delays between application and harvest vary (10 vs. 30 moths).[41] Grazing animals must not be allowed access to grassland or forage land for at least three weeks after the application of sludge.

Measures to prevent post-harvest contamination

The maintenance of cold storage conditions throughout processing, storage, transportation, handling and retailing of fresh produce is important, as this will prevent the proliferation of many mesophilic bacteria (Castro-Ibáñez et al., 2017). Growers and fresh produce processors are required to take adequate measures, as appropriate, and to use potable water or clean water, whenever necessary, to minimise microbial contamination of produce via water (FAO/WHO, 2019a). Disinfection of process wash water is necessary to prevent the transfer of microorganisms through the water between production cycles. The Code of Hygienic Practice for Fresh Fruits and Vegetables establishes several sanitary practices that might be considered as preventive measures to avoid contamination of equipment associated with growing, harvesting and processing (CAC/RCP 1-1969 (FAO/WHO, 2020), CAC/RCP 53-2003 (FAO/WHO, 2017)). HACCP systems are recommended minimise microbiological hazards and measures often include the use of biocides, some of which may select for AMR if their biocidal effect is incomplete (Elekhnawy et al., 2020; Guérin et al., 2021). Fresh produce can be subjected to primary preparation in the field, including cleaning, cooling (e.g. hydrocooling) trimming and coring of raw materials (FAO/WHO, 2008). In some cases, fresh-cut processors use field coring and trimming of lettuce. This removes the external leaves, and then, the lettuce heads are packed in the field.

Other measures related to the control of environmental AMR sources in this sector, such as workers, equipment, wild animals and process water are included in Sections 3.3.1.2 and 3.3.1.4.

3.3.1.2. Measures for mitigation of AMR in the terrestrial animal production sector

General sources/measures

Environmental sources identified in Section 3.1 include air/dust, feed, animals (wildlife, rodents, arthropods) and soil. Inevitably, ARB are disseminated within herds or flocks through contamination of the production environment with faeces. Thus, improved hygiene measures, improved cleaning and disinfection, control of effluents, farm pests and dust as well as sourcing or more careful management of replacement animals are important. Some examples for those specific sources are given below.

Production facility design and management

Farm and building design to facilitate maximum biosecurity, sectioning and cleanability and to minimise stress-related disease can be beneficial for control of both pathogens and AMR (Alarcón et al., 2021).

Manure management systems such as enriched cage or aviary systems or dairy/beef facilities may use manure belts or automated scraper systems to regularly remove the faecal waste (which will end up as manure or slurry). Slatted/partially slatted floors also reduce faecal contact with animals. Manure pits to store animal waste, either beneath slatted housing or in outside facilities, minimising spillage of waste and preventing easy access for other animals, including wildlife, is also important. Further information on best available practices is provided in Commission implementing Decision 2017/302.

Improvement of cleaning and disinfection protocols for livestock housing, overcoming the difficulties in removing persistent bacterial pathogens from animal accommodation is vital, but poorly described for environmental ARB (Boughton et al., 2007; Mannion et al., 2007; Carrique-Mas et al., 2009; Taylor et al., 2009; Heinemann et al., 2021).

Additional hygiene measures for bedding material to avoid contamination of stored bedding (Pope and Cherry, 2000) may also help reduce ARB. Treatment of bedding materials such as wood shavings or chopped straw with organic acids or by biocidal fumigation to reduce bacterial contamination could be a practical option (Solan et al., 2011).

Control of dust through the use of air pollution electrostatic or wet air scrubbers, air outlets fed into sanitary dust traps and filters, UV or ionisation treatment (e.g. using ionising lamps or by loaded fibres) or disinfectant misting of incoming air is possible. For dust within animal housing, fogging without disinfectant is also effective in reducing airborne dust (Ullman et al., 2004), although this could undermine the need to keep the flooring and litter dry. Dust blowing in from other farms can also be reduced by placement of thick, tall Leylandii hedges or shelter belts around the barns; however, this can attract farm pests (Varshney and Mitra, 1993). As dust rapidly becomes diluted over distance, it is best to place animal houses as far apart as is practical (Wood and van Heyst, 2016; Theofel et al., 2020).

Biosecurity

Controlling and managing access of humans into and within production and processing facilities is an important facet of biosecurity. Restrictions/quarantine periods may be applied for people who have visited other farms, have travelled abroad recently or have recently suffered from infectious diseases (Voor In 't Holt et al., 2020). Control of staff and contractors' visits through signing-in procedures, visitor posters, booklets and certificates can raise awareness amongst visitors about the importance of biosecurity. Workflows within farm premises which avoid transfer of ARB within the unit or facilities (van Steenwinkel et al., 2011), hygienic premises (clean toilets, changing rooms, showers), provision of protective clothing (e.g. dust masks; shown protective against MRSA in short term-visits to a swine farm, Angen et al., 2019), boots, good house entry facilities involving a hygiene lock, boot changes and disinfectant boot dips and at least a hand sanitiser in each house should minimise risk (Sibanda et al., 2018; García et al., 2020). Enhanced use of personal protective equipment and contact tracing can be considered for avoiding contact and for eventual tracing back to the introduction of specific emerging resistance into farms and consequently taking measures to reduce spread, as has been used with success against MRSA in Norway (Elstrøm et al., 2019a,b).

Prohibition of thinning would be likely to have a major positive impact on the introduction of unwanted bacteria onto poultry farms (Higham et al., 2018), both on the staff and on catching crates, modules, forklift trucks or broiler harvester machines (Allen et al., 2008).

Motivation of some farm owners and workers requires improvement to ensure that best practice is implemented. Audits and public disclosure of data has been a successful technique to encourage better control of important animal diseases and to reduce AMU (Belay and Jensen, 2020), and could be extended to the results of biosecurity and hygiene audits, along with social science-based methods such as encouraging pride in the job role, peer respect and competition/ranking tables (e.g. being in 'top third' of producers), clear messaging, gaming app-based training and nudging techniques (Gunn et al., 2008; Rose et al., 2018; Rejeb et al., 2021).

Access of production environments to animal species other than the livestock reared on the farm should be limited. Such interventions reduce an important source of AMR introduction. Restricting co-grazing practices limits the risk of transfer of AMR among co-grazed species (Ma et al., 2019). Access of pets such as dogs and cats and wild animals to food-animal areas, feed or bedding stores should be limited as much as possible (Wedley et al., 2017).

Rodent control must be put in place, through proofing and, where required, baiting, although there is an increase in genetic resistance to the most commonly used second-generation anticoagulant baits (Frankova et al., 2019; Buckle et al., 2020). Avoiding spilled feed and clutter, proofing buildings and limiting perching places reduces the attraction and access of birds, while arthropod control requires hygienic conditions such as fresh, dry bedding and removing mortalities promptly (Frosth et al., 2020), alternatives to insecticides, such as carnivorous wasps, beetles or insecticidal fungi plus timely application of larvicidal and knock-down acaricides can be used if needed.

Measures to reduce contamination in feed

Feed production and manufacture should be considered as an integral part of the food production chain. Currently mandated good manufacturing practices (GMPs) and HACCP-based hygiene programmes, especially heat treatment and prevention of recontamination (Tielen, 2009), which are subject to quality assurance and food safety systems, are largely aimed at *Salmonella* (Manning et al., 2006). These approaches should be evaluated for their efficacy at mitigating the burden of spread of other potentially more heat-resistant bacteria (Amado et al., 2014) and for ARGs, and therefore reducing animal and plant exposure. In contrast, the treatments increase the energy required to produce feed, may damage thermolabile feed compounds such as vitamins and proteins or even probiotic additives added to feed.

Measures to reduce contamination in other sources

Possible measures to reduce AMR development linked to the use of waste milk used to feed calves were reviewed by EFSA (EFSA, 2017). Application of thermal treatment is considered to be the most effective method for ARB and pathogen reduction in milk and is increasingly widely used. However, the process has limited activity on antimicrobial residues present in the milk which can still select for ARB in calves and their environment (Aust et al., 2013). Potential environmental risks originating from feeding calves with milk of cows treated with antimicrobials or by disposal of this milk into the environment would require further investigation. Treatment of waste milk for disposal, such as by co-digestion with manure, might be options to be considered, with the additional advantage of increased biogas production (Wu et al., 2011).

Other measures related to the control of environmental AMR sources in this sector, such as manure, as well as different types of water are included in sections (3.3.1.1 and 3.3.1.4).

Measures to reduce contamination during transport and post-harvest processing

Current mitigation measures in the post-harvest environment are based on common general measures focusing on hygiene and good manufacturing practices, which are not specific for ARB/ARGs but could be efficient for reducing or preventing their introduction and spread within the food chain. Although theoretically useful, such measures require validation regarding their impact on AMR (West et al., 2018).

Hygiene and disinfection in transport and lairage (Mannion et al., 2008; Walia et al., 2017) together with management measures such as strict separation of animal batches, which can be extended to slaughter practices (Kudirkienė et al., 2011; Obe et al., 2020) may reduce the spread of ARB in these two stages. For disinfection, the appropriate choice of biocides, their concentration and application rate, validation and regular testing and potentially the rotation of biocide classes are of relevance. These practices can be extrapolated to other points of the food chain.

Both slaughterhouses and processing plants may disseminate ARB and ARGs (see Section 3.1). HACCP programmes (including scheduled slaughter guided by food chain information, FCI) play an important role in the control and reduction of spread of pathogens and thus, they could also help to diminish the risk of AMR introduction into the food chain (Buncic and Sofos, 2012). Procedures such as cleaning and disinfection with hot water, steam or biocides that minimise the survival of surface contamination and dissemination of bacteria to other batches of animals slaughtered on the same day, to workers or the environment during slaughter are important. Use of multi-stage counter-current or hot scald tanks, carcass singeing (for pigs), efficient air handling systems, physical or spatial separation of operations and lines, suitable personal protective equipment (PPE) and hot water decontamination for knives, gauntlets, etc., suitable designed water sprays for equipment and carcases at strategic points along the line, well-adjusted cutting and evisceration equipment, heat treatment of recycled water and air/blast chilling are examples of important considerations (Althaus et al., 2017; Guergueb et al., 2020). Steam plus ultrasound decontamination or rapid surface chilling can also be used to reduce bacterial counts on carcases (Burfoot et al., 2016; Kure et al., 2020). Alternatives for equipment decontamination such as the use of ultrasound (Brasil et al., 2017) cold plasma (Varilla et al., 2020) or thermal ultraviolet systems are new measures for which efficacy for ARB reduction should be supported with data. However, there are *in vitro* studies that report that some new alternative non thermal decontamination methods such as UV light and non-thermal atmospheric plasma (NTAP) decontamination techniques may also lead to selection of resistance to clinically relevant antimicrobials (Álvarez-Molina et al., 2020) although current data is not conclusive.

One-time measures

In very exceptional occasions, culling of animals, followed by microbiological decontamination prior to re-population of the herd, has been used as a means to eliminate the risk of spread of newly emerging resistance into a country or region, e.g. for MRSA CC1 identified in pigs, and sheep related to pig farms, in Norway (Elstrøm et al., 2019a) or the *Salmonella* Typhimurium DT104 clone in Denmark (Alban et al., 2012). Also, in view of the rapid spread of MDR strains in other countries, the UK has taken action to eradicate MDR *Salmonella* Infantis or Kentucky in UK poultry flocks when it occurs, which has been successful to date (Newton et al., 2020).

3.3.1.3. Measures for mitigation of antimicrobial resistance in aquaculture

For this sector, the most important sources identified in Section 3.1. were related to contamination of water and associated sediment, especially via faecal pollution from humans and animals. Additionally, fish feed may serve as a source of contamination. Measures to address water as a contamination source in aquaculture systems are discussed in this section while the EU legislation covering water, as well as broader measures to prevent the contamination of water by ARB, are covered in more detail in subsection 3.3.1.4. Measures to reduce contamination of feed by ARB are similar for feed intended for terrestrial and aquaculture animals and are covered in Section 3.3.1.2.

As described in Sections 3.1 and 3.2, there is limited available data on AMR in European aquaculture systems, but the absence of data does not mean that there are no ARB present within aquaculture environments. Many resistant organisms, including AMR pathogens, originate from human and livestock faecal pollution and remain present in discharged water despite passing through wastewater treatment. Measures to reduce or eliminate ARB and ARGs in wastewater effluents are therefore of particular relevance for the aquaculture sector. In addition to alternatives to AMU, such as vaccines for some types of bacteria and probiotics, a number of measures can be put in place to reduce the occurrence, persistence and spread of ARB and ARGs.

Measures to reduce contamination of aquaculture species and products by ARB in water or sediment

Water has been identified as the main source of contamination of aquaculture species and associated products with ARB. Maintaining good water quality including water with a low (or zero) load of microbial pathogens or ARB and ARGs should therefore be consistently achieved. In closed, land-based systems, the choice of water source is therefore of relevance. E.g. to prevent introduction of ARB into freshwater recirculating aquaculture systems (RAS), ground water could be used as a water source. In open systems, measures should aim to prevent the introduction of ARB and ARGs into the surrounding waterbodies, which can be achieved by improved wastewater treatment and strategies to reduce waste or run-off from terrestrial food animal production systems, or human habitations, that could potentially act as a source of ARB or ARGs into rivers and coastal waters which could then impact aquaculture production systems. In addition to the above-mentioned measures, environmental interventions could be implemented, such as wastewater storage tanks, improved drainage or constructed wetlands aimed at reducing discharge of ARB or ARGs into waterbodies near aquaculture sites (Pazda et al., 2019).

In general, effective environmental management, such as optimal site selection, choice of water source, facility design and effective waste removal (FAO/OIE/WHO, 2006) will contribute to reducing AMR contamination (see Section 3.3.1.4). With regard to optimal site selection, this could include the positioning of aquaculture sites away from areas of wastewater effluent outfalls and intensive livestock production (FAO/WHO, 2019a) to avoid spread of ARB and ARGs into aquaculture production systems and positioning farms sufficiently far away from each other (Henriksson et al., 2018) to prevent transfer of ARB between farms. Attention should also be paid to aquaculture as a source of ARB and ARGs for downstream river catchments, lakes or coastal environments, which may impact terrestrial livestock production systems or facilitate transmission to humans through direct contact with the aquatic environment.

Drying of sediments, liming of ponds and organic waste removal before restocking will reduce carry over of microbial pathogens including ARB (Henriksson et al., 2018). Development of better management strategies to prevent the emergence of resistance gene pools in sediments of aquaculture facilities and to remove already established AMR should be a management objective (Tamminen et al., 2011).

As mentioned in Section 3.1, recirculating aquaculture systems (RAS) are used in intensive farming of both fresh and marine fish species and is an increasingly used biosecurity system (Henriksson et al., 2018). However, antimicrobial residues from feed may accumulate in such systems (Martins et al., 2010) and little is known about the occurrence of ARB and ARGs in RAS (Watts et al., 2017). A recent study from Switzerland found that resistance levels were higher in bacteria of fish from RAS farms compared to farms using flow-through systems (Delalay et al., 2020). Biofilm formation in biofilters of these systems was identified as a potential risk factor for AMR development. Some recent research also suggests that microplastics could be a reservoir of ARB and ARGs in RAS farms (Lu et al., 2019; Zhang et al., 2020c), with microplastics previously shown to increase the lateral transfer of ARGs in aquatic environments (Arias-Andres et al., 2018). Since the use of RAS will increase in the future, research into the occurrence of ARB and ARGs in these systems as well as technical systems that can effectively remove them is warranted. Water treatment and filtration is widely used within RAS systems. Biological filtration, membrane filtration and UV treatment are used to remove excretion by-products and microorganisms entering and developing within the system, but their efficacy in terms of reducing ARB and ARGs requires further investigation. Chemical water treatments that may produce harmful disinfection bioproducts, and biocides that may promote AMR through co-selection or mutation should be avoided (Lieke et al., 2020).

Biosecurity

In addition to measures that specifically address the major sources that have been identified, general biosecurity measures can prevent and reduce the risk of AMR dissemination within aquaculture facilities. Maintaining optimal environmental conditions such as stocking densities, good water quality, proper feeding, high standards of hygiene and vaccination will improve the general health of aquatic animals, improving their ability to withstand disease (FAO/OIE/WHO, 2006), and as a consequence reduce the use of antimicrobials and selection for and spread of AMR.

To reduce disease burden, the use of exclusively disease-free juveniles for rearing is crucial (Henriksson et al., 2018) and implementation of fallowing (gaps in production) between the rearing of different fish cohorts can be introduced, if practical (Wellcome Trust, 2018). Furthermore, in order to exclude disease vectors from aquaculture facilities, bird nets and scarers and barriers for crabs can be used (Henriksson et al., 2018). In the case of open freshwater aquaculture, rodents and other wildlife should also be excluded. Since ARB may be transmitted both from workers to the fish and fish products and *vice versa*, hygienic fish handling by workers is important (FAO/OIE/WHO, 2006).

Sanitation and disinfection

Sanitation and disinfection of aquaculture and hatchery facilities is important to control both vertical and horizontal transmission of infectious diseases (FAO/OIE/WHO, 2006), and this would include ARB. Attention should be given to the ability of some heavy metal and biocide treatments to co-select for AMR or stimulate MGE transfer and caution should be applied to their use if other non-selective (in evolutionary terms) treatments are not available. Heavy metals are used as antifouling agents in aquaculture cages and nets in marine systems and there is evidence that these could also exert a co-selective pressure for AMR (Nikolaou et al., 2014; Watts et al., 2017). Optimal disinfection of equipment such as nets (Henriksson et al., 2018) and processing equipment, may further reduce disease transmission, including ARB, bearing in mind the potential for AMR co-selection associated with certain classes of disinfectants.

Measures to reduce contamination during transport and post-harvest processing

To prevent the spread of ARB and other bacterial pathogens during transport, developing systems for the treatment of transportation water and avoiding contact with other aquaculture animals during transport (EC 2015/C299/04) is important. As for the other sectors, during post-harvest processing good hygiene and manufacturing practices including HACCP programmes would diminish the risk of introduction and spread of ARB and/or ARGs.

3.3.1.4. Measures for mitigation of AMR in water

As previously shown, different types of water (i.e. surface water, wastewater, wastewater treatment plant effluent and reclaimed water) are recognised as important sources of AMR in the food-production environments of all the sectors considered. As water can also be a vehicle for aquatic bacteria and viral particles able to transfer resistance determinants, mitigation measures applied to reduce AMR selection and spread in water environments are recommended (Bürgmann et al., 2018). There is little information on measures specifically targeting AMR in water, with most focused on reducing bacterial and pathogen load. Depending on the types of water and their use, there are several EU regulations that cover water quality (microbial and/or chemical) and therefore could be relevant for mitigation of AMR. Some of these regulations play a major role for the agri-food chain while others are more relevant for aquaculture and the general aquatic environment. EU legislation covering water quality of relevance for AMR mitigation within the context of this opinion is shown in Table 12 and Appendix A. Additionally, in the following text mitigation measures specific to the different waters are reviewed.

Classical water treatment is focused on biological treatment, thermal treatment, filtration processes or chemical treatment of water for effective reduction of bacteria and improvement of water properties. Measures to reduce microbial discharge, such as advanced wastewater treatment are currently being investigated in relation to their effect on AMR. Currently, such treatments are implemented e.g. when discharging wastewater directly to a 'sensitive area' such as bathing waters or shellfish waters, or when reclaimed water is used for irrigation.

Rizzo et al. (2020) reviewed the efficacy of advanced wastewater treatment methods such as ozonation, activated carbon adsorption, chemical disinfectants, UV radiation and advanced oxidation processes (AOPs) with respect to the removal of ARB and ARGs, and their advantages and disadvantages. Bürgmann et al. (2018) reviewed measures for reducing antimicrobials as well as AMR including AOPs such as combinations of ozone, UV and hydrogen peroxide that employ hydroxyl radicals to aggressively attack organic matter, including antimicrobials and other pharmaceuticals. They also considered membrane filtration and reverse osmosis treatment which may even be able to remove DNA implicated in bacterial transformation and acquisition of ARGs. Membrane filtration and reverse osmosis treatments seem to be the most appropriate emerging interventions to reduce AMR; however, efforts should also focus on improvements to conventional wastewater treatment and reduction in raw sewage discharges which have a significant impact on AMR in rivers and coastal waters.

The Norwegian Scientific Committee for Food and Environment (VKM) also recently published an assessment of the impact of wastewater and sewage sludge treatment methods on AMR where membrane processes were identified as a the most promising option for removing ARB and ARGs during quaternary treatment (VKM, 2020). In this report, membrane processes and membrane bioreactors were considered advantageous due to high treatment performance and compact design (VKM, 2020).

UV and ozonation treatment may be used for the purpose of removing chemical contaminants such as pharmaceuticals. They have the potential to reduce bacteria and, to a lesser extent, resistance genes (Rodriguez-Chueca et al., 2019); however, their effect depends on the treatment intensity (Czekalski et al., 2016) which is insufficient to remove bacteria in circumstances geared towards removal of pharmaceuticals (Rodriguez-Chueca et al., 2019). UV radiation used for water disinfection has been shown to reduce the total abundance of ARB but the actual dosages that would be needed for effective inactivation would be very high as treated wastewater still contains high concentrations of particles to which bacteria and extracellular DNA can attach or absorb and thus be shaded from exposure to this radiation (VKM, 2020).

With regard to the plant sector, land used for the production of plant-based foods may require irrigation, with sources being surface or ground water, or reclaimed water from wastewater treatment plants among others. Reducing AMR inputs to food-producing environments from various sources (direct deposition, runoff, sewage discharge, infiltration and lateral flow in shallow soils) and/or from microbial reservoirs (e.g. bottom sediment, bank soils) could be possible strategy for microbiological water quality control. Other measures could be the treatment of water during the storage, between storage and delivery systems, and in the delivery systems (Gil et al., 2015).

A multiple barrier approach is recommended whenever wastewater, wastewater treatment plant effluent or surface water are used in plant irrigation or when wastewater impacts aquaculture (WHO, 2020). Efforts are being made to develop a multi barrier water safety plan approach, which includes the protection of source water at the catchment scale. This ultimately aims to reduce pollution from both human and livestock waste, thereby decreasing drinking water treatment costs at abstraction points. An associated benefit is that downstream water quality in shellfish production and bathing waters will be improved. Interventions include reducing misconnections from sewage systems to surface water drains, reducing combined sewer overflows (CSOs), improving septic tank function, reducing farm run-off through planting of barrier strips around rivers, reducing access of animals to surface waters and ensuring good on-farm practice in terms of slurry and sludge disposal. For human waste, secondary conventional treatment can achieve a 2–3 log reduction with tertiary treatment achieving a 5 log reduction of *E. coli* (Zhang and Farahbakhsh, 2007), so ineffective septic tanks, CSOs and misconnections may have a disproportionately large impact on receiving water microbial and ARB/ARGs load. A recent global survey of wastewater treatment plants, including a large number in Europe, reported a 2.1 ± 0.8 log removal for coliforms in conventional activated sludge treatment, 4.4 ± 2 log for final disinfection and 5.8 ± 0.6 removal for membrane bioreactor treatment (Marano et al., 2020).

In general, improved treatment of wastewater will have a similarly beneficial effect on transmission of water-borne disease, and help to mitigate possible future water shortages due to climate change, but there are energy, resource and land use implications associated with the use of more advanced technologies and extensive constructed wetlands.

Table 12: EU legislation covering water quality of relevance for AMR mitigation

Directive/ Regulation	Objective	Relevance for AMR
Regulation on Water Reuse (Regulation (EU) 2020/741)[(a)]	Comes into force in June 2023. Sets out water quality and monitoring provisions on risk management for the safe use of reclaimed water for agriculture irrigation, and establishes minimum standards for microbial quality.	The Annex II of this regulation refers to the risk management measures which may be needed, and includes AMR as a condition which might require additional effort: '*(B) 6. Consideration of requirements for water quality and monitoring that are additional to or stricter than those specified in Section 2 of Annex I, or both, when necessary and appropriate to ensure adequate protection of the environment and of human and animal health, in particular when there is clear scientific evidence that the risk originates from reclaimed water and not from other sources'.* Such additional requirements may in particular concern antimicrobial resistance.
Drinking Water Directive (Directive (EU) 2020/2184)[(b)]	Concerns the quality of water intended for human consumption and covers water used in the food-processing industry, and has recently been revised. The revised Directive not only sets out stricter chemical and microbiological quality standards but additionally requires Member States to apply a risk-based approach to cover the whole supply chain. Microbiological parameters, which must be complied with, are intestinal enterococci and *E. coli*, and indicator parameters, which must be monitored, are *Clostridium perfringens* and coliform bacteria. Member States are required to undertake additional monitoring of substances and microorganisms for which no standard has been set, on a case-by-case basis, if there is reason to suspect that they may be present in numbers or concentrations which constitute a potential danger to human health.	While AMR is not explicitly mentioned, drinking water must be free from any micro-organisms and parasites and from any substances which, in numbers or concentrations, constitute a potential danger to human health. By 2029, the Commission will have to prepare a report on potential threats to sources of water intended for human consumption and associated health risks from contaminants of emerging concern.
Urban Waste Water Treatment Directive (UWWTD, Directive 91/271/EEC)[(c)]	Has the objective to protect the environment from the adverse effects of wastewater discharges. Most of the measures in place for wastewater treatment aim to control nutrient levels in effluents to avoid adverse effects in receiving waters, such as eutrophication, although conventional technologies can reduce both microbial and chemical concentrations in many cases. There are no direct microbial standards for the quality of wastewater effluent discharged to surface waters set at European level (except for an indirect sum parameter of organic load, the biological oxygen demand (BOD) which, however, does not correlate to microbial load).	The UWWTD is currently subject to an impact assessment, which might lead to a revision of the Directive.[(d)] In this context, the impact of some policy measures, such as upgraded treatment, on AMR propagation is being considered.

Directive/ Regulation	Objective	Relevance for AMR
Bathing Water Directive (Directive 2006/7/EC)(e)	Sets limits for *E. coli* and intestinal enterococci in bathing waters.	
Commission Implementing Regulation (EU) 2019/627(f)	Lays down the conditions for the classification and monitoring of classified production and relaying areas for live bivalve molluscs, in accordance with Regulation (EU) 2017/625, and sets thresholds for *E. coli* in molluscs (mainly bivalves), categorising them from A to C based on numbers of *E. coli* with associated regulations on shellfish sale as a food product.	These regulations indirectly drive water quality in shellfish production areas or mean that shellfish production cannot be situated in waters highly impacted by wastewater from humans and/or livestock.
EU Water Framework Directive(Directive 2000/60/EC)(g) Environmental Quality Standards (Directive 2008/105/EC)(h)	Chemical pollution of water is monitored under the EU Water Framework Directive, with 'Priority Substances' in surface waters being regulated in terms of concentration through the daughter directive on Environmental Quality Standards. Currently there are no antimicrobials listed as Priority Substances, but there are several antimicrobials on the Watch List. Watch list substances should be monitored to a limited extent by Member States, to allow assessment of the EU-wide risk they pose to the aquatic environment and whether they should be set as Priority Substances.	The current Watch List (as adopted by EC in August 2020) includes the beta-lactam amoxicillin, the fluoroquinolone ciprofloxacin, trimethoprim and sulfamethoxazole (Commission Implementing Decision 2020/1161). Previous lists (Commission Implementing Decision 2015/495) included macrolide antibiotics (erythromycin, clarithromycin and azithromycin).

(a): Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 May 2020 on minimum requirements for water reuse. OJ L 177, 5.6.2020, p. 32–55.

(b): Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast). OJ L 435, 23.12.2020, p. 1–62.

(c): Council Directive 91/271/EEC of 21 May 1991 concerning urban waste water treatment. OJ L 135, 30.5.1991, p. 40–52.

(d): Results planned for: Q1 2022.

(e): Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. OJ L 64, 4.3.2006, p. 37–51.

(f): Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls. OJ L 131, 17.5.2019, p. 51–100.

(g): Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L 327, 22.12.2000, p. 1–73.

(h): Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. OJ L 348, 24.12.2008, p. 84–97, with consolidated version available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1603203464448&uri=CELEX:02008L0105-20130913

3.3.2. Potential mitigation measures under evaluation

Here we describe possible measures that could be used to reduce AMR in general or be used in particular sources/sectors. These may include measures to eliminate, prevent/reduce or control risks and dissemination of AMR from food-producing environmental sources.

- Implementation of measures/systems aiming to control (i.e. mitigate), to limit the spread, persistence or the increase of AMR and monitor the frequency or the abundance of ARBs. The impact on AMR due to local or global climate change should be investigated and mitigated where required. Relevant factors include increased growth or survival of human and animal commensals and pathogenic ARB resulting from increased temperature, flooding of agricultural/farm areas leading to increased microbial transport, drought and low water flow rates leading to higher concentrations of contaminants and other impacts on ecosystems, etc. E.g. more frequent extreme rainfall events can be mitigated by better land management. This might include, e.g. zero, tillage cultivation and use of flotation tyres for heavy agricultural machinery, maintenance of effective controlled field drainage, integration of managed woodlands with intensive agriculture, clearance of silted rivers and use of constructed wetlands (Cooper et al., 2019).
- Measures aimed directly at ARB, specific ARG or mobile genetic elements are rarely described; however, the following interventions based on biological methodologies mentioned below could be proposed for potentially applying more targeted control options to diminish the proportion of particular ARB amongst the general microbiota in the future. However, their ecological impact should be assessed.
    - The use of competitive exclusion bacteria (Luyckx et al., 2016) which could reduce acquisition of ARGs or displace ARB from terrestrial production environments. This approach could also be used in animal facilities, particularly for chicks or the newborn/farrowing environment from which the first microbiota is largely derived (Ceccarelli et al., 2017). It may also be possible to use predatory bacteria, such as *Bdellovibrio bacteriovorus*, and/or phage to reduce ARB (Herencias et al., 2020; Hobley et al., 2020; Lewis and Hill, 2020; Premaratne et al., 2021).
    - CRISPR-Cas technology could have the potential to inactivate ARGs in bacterial cells. Utilisation of CRISPR-Cas targeting ARGs and/or MGEs could be used in the future, subject to overcoming regulatory barriers (Pursey et al., 2018). An important challenge to achieve this in a complex environmental microbial community, is the design of an appropriate delivery vector by which the CRISPR-Cas system could be introduced into ARB. This could be achieved through the use of a lytic phage or a (broad host) conjugative plasmid. Preferentially the system is able to remove ARB, and/or ARGs from the microbial community (e.g. by using a lytic phage as vector).
- Measures to reduce factors that promote genetic events leading to the development and/or increase of more environmentally resilient resistant variants. Low levels of certain stressors such as antimicrobials, oxidising agents and certain biocides are known to initiate SOS stress responses in bacteria, increasing their survival potential and, in some cases, their virulence (Baharoglu and Mazel, 2014).
- Better ways to incentivise best hygienic practice amongst food business operators (FBO) managers and staff. Cross-contamination of food by ARB when using insufficiently hygienic practices when handling food items can lead to dissemination of ARB/ARGs, therefore appropriate education and training of managers and staff, as well as suitable auditing schemes and technological solutions to ensure compliance, are expected to improve the implementation of suitable measures.

Monitoring, surveillance and epidemiological studies (longitudinal and sectorial):

Although monitoring and surveillance activities for ARB and/or ARG are not mitigation options as such, conducting monitoring studies targeting AMR in the environment of specific production sectors will generate data potentially leading to focusing or assessing future actions (Hendriksen et al., 2019;

Sims and Kasprzyk-Hordern, 2020). Depending on the objectives pursued,[42] specifically focussed epidemiological studies could be designed and carried out. Considering the different sources identified during the elaboration of this scientific opinion, the implementation of monitoring/epidemiological studies could potentially target:

- Plant-based food:
    - Preharvest: manure, irrigation water, treated sewage sludge, soil.
    - Post-harvest: crops, surfaces of processing equipment, process water.
- Terrestrial animals:
    - Preharvest: surfaces (animal accommodation and handling areas, lorries, livestock markets, knackers yards, milking parlours/milk, filters), livestock lorry wash centres, manure, run-off and surface water accessible by the animals, dust and air, soil and pasture, feed, feed processing dust (feed mill), wildlife (including arthropods/rodents/wild birds), workers.
    - Post-harvest: slaughter/processing plants/ABPs producing plants: workers, process water/ drains, equipment surfaces, slaughter waste.
- Aquaculture:
    - Preharvest: water, fish/bivalves/shrimps, sediments, workers, feed, juveniles for rearing.
    - Post-harvest: workers, processing equipment/wash-water, surfaces, fish/bivalves/shrimps.

For aquaculture, see also EFSA recommendations in Section 1.6.

Recommendations for studies that would deserve priority are provided in Section 3.4.

3.3.3. Impact of the options to mitigate the emergence and spread of antimicrobial-resistant bacteria and resistance genes

The impact of the measures mentioned above on the reduction of ARB transmission or ARG transfer within source environments cannot be fully assessed, as there are very few available studies on ARB and ARG transmission in the environment (Lima et al., 2020), particularly in the EU. However, a number of measures have been shown to be effective for the reduction of pathogens, which will also have an impact on the occurrence and level of ARB. Evidence does exist for the effectiveness of wastewater treatment, especially filtration methods, for reduction of ARB and ARGs, with standard treatment strategies reducing discharges of ARB (Marano et al., 2020) although some studies have indicated increased prevalence of AMR in effluents, due to less efficient removal or selection for ARB and/or increased rates of HGT, despite overall reductions in absolute numbers of ARB (Wang et al., 2020b; Zhang et al., 2020d).

For most mitigation options the impact is dependent on environmental sources and the specific AMR present, and therefore, the results may vary in different settings or due to local conditions. Thus, before implementation, it is necessary to evaluate the applicability of measures more robustly, including in the specific local situations where they will be used.

We have gathered information from the literature on specific mitigation options by sector and sources as well as their advantages and disadvantages; however, the data are scattered across studies with different methods and aims. Therefore, we consider the prediction of the impact of such measures to be very complex, resulting in large uncertainties linked to lack of standardised monitoring on AMR sources and gaps in existing knowledge (Table D.1, Appendix D). In general, measures based on improved biosecurity and hygiene throughout the food chain are also likely to provide better control of animal and food-borne zoonotic pathogens. However, increased heat treatment of animal feed or waste milk/colostrum may reduce the nutritional or immunological value and hot water washing of processing equipment and carcases may generate steam and condensation that results in other microbiological and operational problems, as well as resulting in greater use of energy and water. More frequent use of biocides, or increasing their concentration, may control ARB more effectively, but could also lead to increased exposure of workers and greater environmental chemical pollution, and some biocides can co-select for AMR. Improved control of wildlife vectors may also risk reducing the diversity of wildlife in rural areas and biocidal products such as rodent baits can also affect non-target species,

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[42] e.g. acquisition of knowledge about the relative importance of sources and transmission of ARB/ARGs, assessment of trends in relation to climate change, changes in regulatory policy or industry codes of practice or food production methodologies, assessment of the impact of specific mitigation measures, assessment of the probability of downstream exposure of food and consumers from different elements of the food production environment.

particularly birds of prey. Failure to effectively implement complementary elements of a control programme can also undermine the whole programme, e.g. thinning of broiler flocks largely negates the preceding biosecurity measures and failure to control rodents or litter beetles means that bacteria can by-pass cleaning and disinfection measures. It is therefore very important that a careful holistic approach is taken for any interventions, particularly those that may impact the environment.

3.3.4. Concluding remarks on mitigation measures

- Apart from prudent AMU, the proper implementation of general measures focused on good hygiene practices and biosecurity to prevent and/or reduce the occurrence and transmission of animal and food-borne pathogens will mitigate ARB. These measures are the most important ones applicable for all the food-production sectors investigated, both at pre- and post-harvest, and are covered by EU Legislation and industry codes of best practice.
- Biological methodologies that focus specifically on the reduction/elimination of ARB in the food production sectors, such as CRISPR-Cas, phages or predatory bacteria are in the early phases of research and development in the AMR field. More data on the efficacy and potential risks of these systems are required before specific recommendations can be made on their utility.
- Activities at production stages which can widely disseminate large numbers of ARB and ARGs in the different production sectors are a priority for intervention. For all sectors, reducing faecal microbial contamination is a priority.
    - For the plant-based production sector, measures reducing the bacterial content of manure (e.g. by composting or anaerobic digestion), sewage sludge (e.g. by heat or chemical treatment such as liming) and irrigation water (see below) should be considered.
    - For terrestrial animals at the farm level, preventing transmission from other animals (e.g. by control of rodents, arthropods and wild birds), dust (e.g. by traps or filters), feed (e.g. heat or chemical treatment) or surface run-off water, is relevant. Cleaning and disinfection of equipment and surfaces, in particular to remove faecal material and bacteria, together with hygienic procedures for workers should be properly implemented.
    - For aquaculture systems, high microbial water quality should be assured by reducing contamination by human faecal waste (e.g. wastewater effluents) and run-off from terrestrial animal production systems. Reducing AMR contamination of fish feed should also be a focus of attention.
    - For post-harvest stages, the implementation of food safety management systems is currently the main mitigation and preventive strategy to minimise the risk.
- Mitigations directed to prevent ARB and ARGs in different water sources (e.g. irrigation water, surface water and fresh and marine water of aquaculture) include:
    - Some advanced wastewater treatment technologies such as membrane filtration or reverse osmosis systems have been recognised as an effective approach to remove ARB/ARGs in full-scale WWTPs and have the added advantage of removing antimicrobial residues.
    - Attention should also focus on reducing current raw sewage discharges and on improving conventional wastewater treatment (e.g. upgrading secondary treatment plants to tertiary treatment standards).
    - A multiple barrier approach to protect plant production and aquaculture, including low impact approaches (e.g. constructed wetlands), combined with improvements to existing and more advanced processes for urban wastewater treatment, is recommended.
- There are few studies on the efficiency of general mitigation options on ARB/ARG elimination. Available studies are supported on general bacterial contaminants.

Regarding the advantages and disadvantages:

- More frequent use of biocides, or increasing their concentration, may control ARB more effectively, but could also lead to increased exposure of workers and greater environmental chemical pollution, and some biocides may co-select for AMR.
- Proper implementation of general measures focused on good hygiene practices, biosecurity and food safety management systems are also likely to provide better control of food-borne zoonotic pathogens, in addition to ARB, but may increase the use of water, biocides and energy.

- Biological methodologies may reduce persistent pathogens, but their ecological impact should be assessed.
- Improved treatment of faecal waste will reduce transmission of faecal pathogens but increases storage and equipment resources requirements and may reduce the fertiliser value.
- Preventing transmission from other animals (e.g. by control of rodents, arthropods and wild birds) will also reduce disease transmission but may also risk reducing the diversity of wildlife in rural areas and biocidal products such as rodent baits can also affect non-target species, particularly birds of prey.
- Improved heat treatment of feed will reduce the risk of ARB and pathogen transmission, but can reduce its nutritional quality, damage added vitamins and probiotics and utilise more energy.
- Improved treatment of wastewater will have a similarly beneficial effect on transmission of water-borne disease, and help to mitigate possible future water shortages due to climate change, but there are energy, resource and land use implications associated with the use of more advanced technologies and extensive constructed wetlands.

3.4. Knowledge gaps and research needs

This section will discuss the knowledge gaps influencing the assessment of the role played by the environment in the emergence and spread of antimicrobial resistance through the food chain and identify related research needs.

There are large numbers of publications and reports that describe the occurrence of AMR in food production environments and the wider environment, including wildlife, and discuss its relevance and potential for mitigation (Manaia et al., 2020; Torres et al., 2020; Kumar et al., 2021; Ofori et al., 2021). AMU is reported as the main driver for the emergence of clinically relevant AMR (Spruijt and Petersen, 2020) followed by transmission or 'contagion' (Collignon et al., 2018). The local and global dissemination of ARB and ARGs by multiple pathways, such as the disposal of waste materials containing antimicrobials, international trade in breeding animals carrying ARB, contaminated food and feed products and global travel is also of high importance (Duran and Marshall, 2005; AbdelRahman et al., 2020; Baloch et al., 2020; Krzeminski et al., 2020). As sanitation and hygiene standards decrease, the risk of transmission between the environment, animals and humans increases, which is a driver of AMR prevalence in human infections. This is supported by the fact that in low- and middle-income countries (LMICs), AMU is not the main driver of AMR, instead contagion (i.e. transmission) is regarded as the most important factor (Collignon et al., 2018). Even in high income countries, environmental transmission is predicted to be a contributory factor to maintaining ESBL-producing *E. coli* and plasmid mediated AmpC-producing *E. coli* carriage in humans (Mughini-Gras et al., 2019).

There is a need for a deeper insight into AMR in a 'One Health' and 'Farm to Fork' context. Requirements include quantitative assessments that enable estimation of the impact of food production and the wider environmental dissemination pathways that directly affect livestock, food and human populations (Léger et al., 2021). This should take into account the effect of future changes in production processes. These may result from factors, such as changes in antimicrobials and heavy metal use for food animals (Turcotte et al., 2020), climate change, expansion of production and food/feed exports from third countries (Silveira et al., 2021), on-going agricultural intensification, vertical/hydroponic plant production and increasingly widespread consumption of meat-free alternative foods (Baekkeskov et al., 2020; Reverter et al., 2020).

The environment, and non-medicated food animals, wild animals and crops are subject to ARB and antimicrobials contamination. It should also be remembered that the natural environment is a reservoir of AMR, and environmental microorganisms are the original source of most antimicrobials and associated resistance mechanisms (Laskaris et al., 2010; Forsberg et al., 2012). The relevance and relative importance of the sources of environmental contamination, and the fate of contaminants in terrestrial and aquatic environments is often poorly understood. Furthermore, the impact of environmental contamination at different stages of the food chain resulting from ARB, ARGs, MGEs or cell-free DNA (Woegerbauer et al., 2020) on the occurrence of ARB in food animals and foods is largely unknown. Likewise, the relevance of environmental pathways via food production to human infections, even for known zoonotic organisms, but especially for commensal bacteria and transferrable ARG, is not clear (Dafale et al., 2020). As far as we know, there have been no published studies that have attempted to quantify or rank these environmental aspects, although there are descriptive studies that report the occurrence of food-borne transmission of pathogens, such as *Salmonella* and

Campylobacter originating from environmental contamination, including some strains which can be resistant to important antimicrobials (EFSA BIOHAZ Pane, 2019b, 2020; Lynch et al., 2020; EFSA and ECDC, 2021a).

The published European research concerning food-production, including the primary production and processing environment, that has been reviewed for this opinion, has been very much influenced, and biased, in favour of topical aspects of public health and scientific relevance. These include ESBLs or fluoroquinolone resistance in Enterobacterales and/or *Campylobacter* in poultry and dairy production or LA-MRSA in pigs. Some studies have also searched databases, or conducted limited field studies for newly emerging resistances that have been initially reported in animal production outside the EU, such as carbapenem and *mcr*-related transferable colistin ARGs (Bennani et al., 2020; Irrgang et al., 2020; Caffrey et al., 2021; Jochum et al., 2021; Ruiz-Ripa et al., 2021). Less well-researched or readily culturable organisms, such as *C. difficile*, may however also be relevant (Heise et al., 2021).

As identified in other reports on the evolution and transmission of AMR in the environment (WHO, 2015; Larsson et al., 2018; Topp et al., 2018), and reinforced within the current Scientific Opinion, large data gaps relating to the food-producing environment remain, including at the EU level. The research that has been conducted so far has focused mainly on the animals themselves at farm or slaughter level, sometimes including the presence of ARB or ARGs in possible environmental sources (such as wild animals or dust). Even if samples have been taken from environmental matrices as part of these studies, this has largely not been done in a systematic way that is comparable between studies in terms of sampling sites, sampling methodology, number of samples or tests used to isolate, detect or quantify varying types of ARB, genes or MGEs. Studies in the aquaculture sector mainly focus on AMR in the fish/shellfish themselves and in most cases the source is assumed to be the growth medium i.e. water, which is an increasing cause for concern in terms of AMR-related pollution (WHO, 2021).

Little EU data is available on the role of feed as a proven source of ARB/ARGs in food animals, even though contamination and recontamination by relevant bacteria (without mention of AMR) is reported (Munoz et al., 2021). Data is also minimal on the effectiveness of feed treatments for reducing AMR contamination (Gosling et al., 2021).

In published reports on AMR, data is often summarised and consolidated so that full granularity is not achievable, e.g. lack of reporting of MDR profiles of individual isolates. The WHO discussion document on AMR priority research needs (WHO, online) focuses mostly on matters relating to usage of antimicrobials and alternatives to minimise usage, which have already been covered in the EMA/EFSA RONAFA Opinion (EMA and EFSA, 2017), so this section focuses on aspects relating to the environment, in keeping with the overall theme of the opinion.

More structured, standardised, repeatable and targeted studies and more efficient methodologies to investigate the ecology of ARB and ARGs in the microbiome of food animals and sources of AMR for the food production environment are required (Cao et al., 2020; Feye et al., 2020; Johansson et al., 2020; Lee et al., 2020; Lima et al., 2020), including the relevance of biofilms (Trampari et al., 2021). Scientific approaches need to move beyond surveys that simply identify ARB in the environment towards systematic and harmonised hypothesis-driven investigations that definitively identify sources of AMR and selective agents for food-producing environments, providing data that can be used to assess the risk to the food chain and public health. Such work should also identify interventions that can interrupt environmentally mediated pathways of AMR selection, acquisition and transmission and evaluate whether current and enhanced management practices and technologies to minimise environmental AMR contamination, especially for management of waste faecal material and water, can lead to desirable outcomes (Checcucci et al., 2020; Goulas et al., 2020; Mencía-Ares et al., 2020; Ricker et al., 2020; Costa et al., 2021; Pereira et al., 2021; Radu et al., 2021; Youssef et al., 2021; Zhou et al., 2021).

New methodologies to help elucidate the complexity of the environmental metagenome are being developed and applied (Gupta et al., 2019; Li et al., 2020; Miller et al., 2020; Stanton et al., 2020; Sun et al., 2020; Xu et al., 2020; Zhao et al., 2020; Liu et al., 2021; Zhang et al., 2021). There are, however, few studies that have used optimised sampling and analytical methodologies to identify and quantify the full complexity of the environmental resistome in a representative way (Agunos et al., 2021; de Almeida Kumlien et al., 2021; Strange et al., 2021), particularly in food production settings. There is therefore a need for increased use of well-structured metagenomic-based surveillance and research at farm and post-harvest level in Europe (Duarte et al., 2020) to complement sensitive traditional selective bacteriological approaches that are still widely used and harmonised via international standards (Mok and Ang, 2016) and to assess the relevance of the large body of non-EU literature for the EU situation. It is also desirable to better link the monitoring, occurrence and reporting of AMR in animals and food with cases of human infection involving ARB (ECDC, 2020) using systematic epidemiological research and attribution studies. These studies are complicated by the fact that many pathogens of concern are opportunists and do not necessarily cause infection at the time of exposure or transmission. Therefore, colonisation, virulence potential linked to AMR and heavy metal resistance and potential for gene transfer, as well as relevance to human infection should also be considered as an end point for epidemiological studies (in addition to infection) as part of overarching One Health-based studies (Hernando-Amado et al., 2019; Findlay et al., 2020; Johanns et al., 2020).

The broad topic areas considered to require further systematic investigation at EU level are:

- The relative contributions of different sources of entry of antimicrobials, ARB, ARGs, MGEs and cell-free DNA into the food-producing environment;
- The role of the food-producing environment, including the impact of anthropogenic inputs, such as heavy metals, biocides or contaminated water, on the selection, evolution and persistence of AMR.
- The overall human and animal health impacts caused by the contribution of exposure to ARB originating from the food-producing environment in comparison with sources directly relating to food animals themselves;
- The effectiveness of current disease and hygiene control measures in controlling introduction and persistence of ABR in the food production environment and evaluation of improved measures, where required;
- The ability of technological, social, economic and behavioural interventions to incentivise improved compliance with best practice to help mitigate environmental AMR in the food-producing environment;
- The likely influence of external factors such as climate change and changes in policy or industry practices on the occurrence of AMR in the food production environment and the extent to which this can be mitigated.

The specific elements within these topics are considered in more detail in Table 13.

Table 13: Data gaps and research needs on the role played by the food-producing environments on AMR

	Data gaps	Recommendations for research
General	• What is the quantitative contribution of the food production and food processing environment to the occurrence and burden of human disease relating to the relative abundance of specific ARB and ARGs?	• Longitudinal studies in food-producing settings and human populations in the EU to investigate temporal associations between the occurrence of specific ARB and ARGs over time, including the role of food production environments in the emergence of novel ARGs in the human microbiome. • Field studies/baseline surveys to assess the distribution and to investigate trends in ARB, ARG and MGE prevalence and diversity in the food production environment designed to inform potential monitoring activities. • Defining suitable harmonised environmental sampling strategies and indicators to assess AMR trends in an efficient way. • Validating suitable analytical tools (including their sensitivity and specificity) to define the complexity of the food-producing environmental microbiome, resistome and mobilome and their suitability for monitoring programmes. • Determining the baseline levels of AMR in food production environments that are subject to different levels of anthropogenic pollution. This knowledge can then be used to establish the significance of anthropogenic impacts on environmental reservoirs of AMR and to inform suitable monitoring and control standards. • Determination of the extent of interspecies transfer of AMR (i.e. between plant, animal, human and environment associated organisms), including internalisation of antimicrobials and ARB/ARGs within plants. • Studies to determine the disease burden associated with AMR originating from the food production environment in different animal and human populations. • Quantitative microbial risk assessment of specific well-defined hazards, supported by the integration of large data sets derived from complimentary microbiological and epidemiological approaches to help assess the public health impact of environmental AMR and potential mitigation options.

	Data gaps	Recommendations for research
Sources	• What are the quantitative contributions of the different AMR sources in the food production environment identified in this opinion? e.g. manure, slurry or sewage sludge, contaminated feed, bedding, air/dust or water, outdoor access, wildlife vectors, replacement animals, to contamination of food production environments and the food chain by ARB	• Studies focused on European food production systems which analyse the environment in detail by validated sensitive, quantitative, microbiological methods, supported by cutting edge laboratory methodology based on NGS/metagenomics and powerful bioinformatic analysis (e.g. machine learning). This will help to characterise and quantify the introduction of AMR into food environments, identify ARB/ARGs and to answer some of the data gaps raised, such as attribution and ranking of food and related environmental AMR sources in terms of public health impact, role of free DNA, phage and MGEs in the spread of AMR in the food chain. • Defining the impact of manure/slurry, dust, natural waters, run-off/wash/reclaimed/wastewater from farms and urban or industrial developments and sewage sludge on the microbiome of agricultural land and natural waters, plant products and terrestrial and aquatic farmed animals. • Defining the relative contribution of wildlife to the dissemination and persistence of AMR in food production environments. • Investigation of AMR in animal feed ingredients and compound feed production. • Exposure experiments using naturally contaminated environmental materials to define the colonising dose for AMR organisms in food animals. • Studies on the occurrence and survival of ARB and gene transfer in different types of farm environments, faecal waste, wastewater and surface/ground water • Structured and targeted studies to investigate the ecology of ARB and ARG in the livestock processing environment by means of microbiome and quantitative culture-based mapping of the input materials, the processing environment, final products and waste materials.
Bacteria and genes	• Which ARB are best able to persist or multiply in the food-producing environment to an extent that can lead to infection/colonisation of food animals or contamination of crops or processed foods at a level that threatens human health? • What is the genetic basis for 'fitness', in relation to persistence of ARB in the production environment throughout the food production chain?	• Defining the adaptive mechanisms that organisms possess to facilitate persistence of resistance in the food production environment, e.g. increased colonisation proficiency, stress response mechanisms, biofilm formation and acquisition or mutation of virulence genes and their expression in environmental niches. • Investigation of the role of clonal dissemination and persistence of pre-existing ARB in the production environment, versus resistance

	Data gaps	Recommendations for research
	• What is the relative quantitative contribution of different gene transfer mechanisms and MGEs to the burden of AMR in the food production environment and transfer of ARGs to human bacterial microbiota? • Even though most human AMR infections are thought to be caused by bacterial strains associated with the hospital and community environment, which ARB and ARGs in the food production environment significantly contribute to the emergence and spread of AMR in these human-adapted pathogens? • What is the significance of cell-free DNA and phage carrying ARGs in environmental reservoirs such as wastewater, surface water and manure, in relation to acquisition of AMR by bacteria in food animals and humans?	selection and transfer of MGEs, including the relevance to emergence of novel ARGs in human commensals and pathogens. • Investigation, using suitable standardised models and field investigations, the role of cell-free DNA and phage in the persistence and dissemination of AMR in the food production environment.
Risk factors	• What is the role of the different risk factors identified in this opinion on the selection, emergence and persistence of ARB and ARGs, particularly those of critical importance for human therapy, including emerging novel resistance mechanisms originating from the food production environment? In particular, the relative contribution of persistent farm contamination, agricultural pollution or contamination originating from human sources, on the food production environment requiring quantification? • What is the impact of different farm management systems, such as intensive, organic, 'antibiotic-free', free-range, on the occurrence, nature and magnitude of AMR in the production environment? • What is the contribution of global trade (workers, animals, feed ingredients, etc.) to the dissemination of AMR through the food production environment? • What is the impact of the use of heavy metals, biocides and pesticides in food animals or the food production environment on the selection for, or persistence of ARB and ARG and promotion of gene transfer? • What is the expected influence of climate-related environmental changes on AMR in the food production environment? • What could be the expected influence on AMR of agricultural policy changes (e.g. the EU 'Green Deal') and the development of the 'Circular Economy'? How can the impact of such changes on AMR be most effectively monitored and managed?	• Studies to obtain a better understanding of the impact of environmentally relevant concentrations of antimicrobials and other potentially co-selective chemicals on environmental microbiology and AMR relating to food production. • Defining the impact of outdoor access animal production and other production systems on the occurrence and persistence of ARB and ARGs through analytical cross-sectional surveys and intervention studies. • Investigation of the influence of international movement of animal feed ingredients, replacement animals and farm staff on the introduction and dissemination of AMR. • Trend analysis linking findings of surveillance and epidemiological studies with climate, farm management and regulatory changes.

	Data gaps	Recommendations for research
Mitigations	• What is the quantitative efficacy of existing production quality and hygiene regulatory and industry codes of practice on the occurrence and persistence of ARB and ARG in the food production and processing environment? • What is the impact of AMU reduction programmes on the occurrence and persistence of AMR in the food-production environment? • What are the most effective and readily applicable methods to treat faecal, processing and water-based wastes from urban, animal production and food processing sources to minimise, or ideally prevent, dissemination of ARB, MGEs and ARGs into the food-producing and wider/natural environment? • What animal feed treatments can be used to eliminate ARB contamination of feed ingredients rations, and to minimise recontamination of compound feed? • How can AMR contamination and cross-contamination of carcases/meat that occurs via the slaughter and processing environment, especially during poultry slaughter, be most effectively controlled? • Which cleaning and disinfection protocols are most effective for elimination of persistent ARB in food production and processing environments, and is their efficiency similar for animal or zoonotic pathogens and ARB? • What educational, technological, social, economic and behavioural interventions would be likely to be effective to motivate farm and food business operators to consistently implement best practice to help mitigate the emergence and spread of AMR via food and the production environment? • What measures can be put in place to reduce any negative impacts of climate change, e.g. the increasing occurrence of periods of extreme hot weather, drought, extreme rainfall events, flooding, extension of the range of wildlife vectors on dissemination of ARB and ARGs in food-production environments? • Which mitigation measures can be used that can specifically target ARB or ARGs?	• Validating that guidelines for hygienic practices, such as industry guidance documents, codes of practice and assurance schemes that are aimed at controlling animal and zoonotic pathogens are sufficiently protective with respect to introduction and persistence of ARB in the food production environment. Suitable indicators to monitor the effectiveness of these practices should also be defined. • Optimising control of surface and airborne contamination and cleaning and disinfection programmes for ARB in animal housing, hatcheries, abattoirs, processing, etc., in relation to international standards for disinfectant approval testing – including assessing alternatives to biocides such as predatory bacteria and steam heat. • Validating the effectiveness of methods for treating manures, slurry/sewage sludge, reclaimed water in terms of a meaningful reduction or elimination of ARB and ARGs. • Development and validation of treatment/mitigation strategies for natural water sources (including surface water) used for aquaculture production and for irrigation and livestock drinking water. • Optimisation of treatment of animal feed to control ARB. • Studies to determine the impact on environmental ARB of the use of biocides in food animal production systems, transport, lairage, slaughtering (including scheduled slaughter based on food chain information in the case of important resistances detected at farm level) or food processing plants on the occurrence of AMR in the food chain and the effect of remedial interventions. • Intervention studies linked to the trend analysis studies relating to climate change mentioned above. • Defining whether the reversal of resistance, which can occur upon termination of AMU, relates to the loss of the resistance determinants or the replacement of resistant bacteria with susceptible bacteria of the same species in the environment. • Validation of specific measures that may be applied in the near future to reduce or eliminate ARB, ARG and minimise gene transfer.

	Data gaps	Recommendations for research
Sector specific		
Plant production environment	• What is the quantitative contribution of irrigation water and fertiliser of faecal origin contaminated by ARB and antimicrobials (including heavy metals and agrichemicals) to the occurrence, transmission and levels of ARB/ARGs in soils/growth matrices and on plants?	• Defining the time window of risk for the occurrence and persistence of environmental AMR following cessation of anthropogenic contamination or AMR-selective factors, based on suitable criteria relating to survival of organisms, potential for growth of ARB or return to background levels of ARB or ARGs, providing evidence for suitable delays between manure application and the harvest of crops destined for human consumption.
Terrestrial animals: *Poultry and pig production environment:*	• What is the quantitative contribution of environmental persistence of ARB in breeding farms, hatcheries and commercial pig and poultry farms to the dissemination and persistence of AMR within the production chain?	• For poultry and pigs – a large cross-sectional study of the whole breeding and production pyramid environment is required to assess the distribution of AMR indicators and risk factors relating to contamination of the production environment and animals/products derived from that environment. This should be followed by a statistically meaningful intervention study using quantitative culture and metagenomics techniques.
Terrestrial animals: *Cattle production environment:*	• What is the importance of AMR in the cattle production environment, including on-farm processing equipment, in relation to AMR contamination of raw milk or artisanal dairy products? • What role does the disposal of waste milk containing antimicrobials and ARB into the environment play in the selection and transmission of AMR?	• Epidemiological and intervention studies specifically targeting raw milk products and waste milk management.
Aquaculture production environment:	• What measures are most effective to help safeguard production systems from 'upstream' AMR from human and animal faecal pollution, especially in the context of water contamination? • What is the relative contribution of fish, and particularly bivalve shellfish, to AMR transmission to humans given that they filter large volumes of water and can concentrate microbial pathogens from upstream contaminated water? • Can AMR surveillance be introduced to aquaculture settings, similarly to the ones in place in terrestrial animals for ARB and ARGs of public health/clinical relevance or does aquaculture need different sampling methods, indicator species, interpretive criteria of resistance (e.g. ECOFFs), genes or even different analyses? Will the metagenomic analysis of the cultivation water be sufficient or is an additional analysis of cultured organisms necessary?	• Epidemiological and intervention studies, and surveillance, using the principles mentioned above, specifically targeting these important aspects of aquaculture. • Definition of the most relevant bacterial indicators and sample matrices for aquaculture, and suitable standards for prevalence, types and levels of AMR in these indicators.

3.4.1. Priorities for future research and surveillance

Despite a large number of studies that have investigated the occurrence of ARB and ARGs in food production, the environmental aspect is not sufficiently researched and, to the best of our knowledge, there is insufficient data to support a specific assessment of the quantitative impact of contamination of the EU production environment on the contamination of foods or threats to public health foods or public health. The most detailed food production environmental studies have been carried out in situations where antimicrobial use or production methods and environmental protection laws, as specified by EU Legislation standards, do not apply. To fill those data gaps, the main research and surveillance priorities would be:

- Comprehensive, integrated studies, linked to One Health initiatives and harmonised environmental AMR monitoring strategies by means of specific focal environmental monitoring or surveillance points are needed. Foci for surveillance studies could include: faecal waste (e.g. manure, sewage sludge) or processing waste, dust from feed mills and animal housing, wastewater from such enterprises, wildlife, irrigation water, run-off, surface water associated with agricultural areas and bivalve mollusc filter feeders and aquaculture fresh/and marine water. Samples taken from surfaces, drains, equipment and workers associated with primary production, slaughter and processing premises, as well as surface samples taken before and after cleaning and disinfection would help characterise the distribution and persistence of contamination. This surveillance, using sensitive and standardised sampling/test methodologies, is required as an urgent priority at EU level to help close data gaps and to properly assess the relative importance of different ARB/ARGs/MGEs in the food production and processing environment. This data will also help clarify the relative importance of the various sources and pathways for transmission and persistence of AMR that apply to the different primary production and processing sectors.

  Within this topic area, the immediate priorities are to:

  - *optimise suitable sensitive and readily standardised culturomics/genomics-based detection methods for currently important and emerging ARB/ARGs.*
  - *define effective sampling strategies for different production environments that will facilitate a meaningful assessment of the occurrence, distribution and level of ARB/ARGs and, based on these studies, to identify efficient sampling frames, sampling points.*

- Long term longitudinal cohort studies on emerging and more widely established high priority ARB/ARGs, using advanced but standardised quantitative microbiology and genomic/metagenomic based epidemiological methodologies in different representative countries within the EU, as well as studies involving environmental exposure of food animals in order to assess the biological relevance of different aspects of environmental contamination.

  - *Within this topic area, the most urgent priority is to focus on case studies on the occurrence and dissemination of emerging bacteria with highest priority ARGs (e.g. relating to carbapenems, tigecycline, isoxazolidinones, colistin) within food production environments and their dissemination into the wider/natural environment via waste, run-off, etc.*
  - Longer term studies would focus on more commonly occurring highest priority resistances, e.g. to extended spectrum cephalopsporins and fluoroquinolones.

- The concentrations of potentially selective and co-selective residues in manures, sewage sludge, irrigation water and aquaculture environments should be further studied to facilitate risk assessment of the role that heavy metals, biocides or excreted antimicrobial residues play in selection for AMR in the environment of food production systems and wider/natural environments impacted by them.
- Effective and practical mitigation methods should be investigated, including assessing the impact on ARB of current biosecurity and hygiene-based control programmes for zoonotic bacteria and animal disease and environmentally friendly water treatment methods such as those based on constructed wetlands or solar treatments.

  - *Immediate priorities within this work should be assessing and developing validated methods for disinfection/decontamination aimed at highest priority ARB in the production environment, heat treatment conditions for animal feed and treatment of faecal waste and*

  - *wastewater used for fertilisation/irrigation or processing crops. Such studies would be expected to yield the most rapid benefits and could be linked with the epidemiological studies mentioned above.*
  - Assessing the role and control of potential wildlife vectors.
  - Further development and validation of novel methods to control AMR dissemination in the environment (e.g. predatory bacteria, bacteriophage, competitive flora, CRISPR-based technologies, etc.).

- Better ways to incentivise implementation of best hygienic practice amongst FBO managers and staff are required to help minimise persistence and dissemination of ARB and these should be investigated using social science-based methodologies, as implementation of suitable control measures is often suboptimal. This work could be delayed until more evidence of effective methods for control of AMR is gained through the studies mentioned above but would still be of immediate value to help reduce food-borne zoonoses and animal disease.
- Longer term studies, using harmonised methodologies, including monitoring strategies, developed in studies described above, could be linked to assessment of the effect of regulatory and climate changes, e.g. Regulation (EU) 2019/6, the 'Green Deal', 'Circular Economy' and initiatives to reduce AMU in food animal production, to conserve water and maximise recycling of natural resources and to maximise the efficiency of food production for an expanding global population.

3.5. Conclusions

ToR1: To identify the main environmental sources and transmission routes leading to the contamination of foods of animal and non-animal origin with antimicrobial-resistant bacteria and/or resistance determinants.

AQ1a. What are the environmental sources and transmission routes for antimicrobial-resistant bacteria and resistance genes for the different food production sectors identified?

- Transmission routes for plant-based food involve the introduction of faecal matter of animal and human origin through fertilisation and irrigation. Other potential sources include soil, dust, farm animals, wildlife, arthropods, workers, contaminated equipment and process water.
- For terrestrial animal production, ARB and ARGs have been found in the following potential sources: feed, farm workers/visitors, rodents, wildlife, arthropods, surface water and dust/air, soil and equipment. Introduction of ARB from these sources into the food production environment may occur.
- For aquaculture, water is a transmission route, with ARB/ARGs in human and animal faecal material as the source. In addition, feed can also be contaminated by ARB and ARGs and water is a source of indigenous aquatic ARB.
- Post-harvest contamination can occur during transport or in the slaughter or processing environment via equipment, personnel and aerosols.
- In addition to the direct introduction of ARB into food production environments, the wider environment itself is a reservoir of ARGs, which may be mobilised to animal and human commensals and pathogens and subsequently established in the food production environment. In the presence of antimicrobial residues, enrichment of these potentially novel resistance determinants may occur.

AQ1b. What is the importance of the different sources and transmission routes of antimicrobial-resistant bacteria and resistance genes?

- The main transmission route for plant-based foods consists of the introduction of faecal material of human and animal origin through fertilisation and irrigation.
- For terrestrial animals, limited circumstantial evidence points to feed and, to a lesser extent, humans, as important sources for introduction of pathogenic bacteria, and therefore possibly for ARB and ARG, into terrestrial animal farms. For other potential sources the published evidence did not allow assessment of their importance.

- For aquaculture, the main transmission route consists of water, with human and animal faecal material as important sources.

For both AQs

- Studies demonstrating and quantifying the introduction of ARB and ARGs from the food-producing environment to the food chain are limited or absent. However, there are studies demonstrating the presence of ARB/ARGs for all sectors and most sources and evidences for the introduction of pathogens from certain sources into food production sectors.

ToR2: Among antimicrobial-resistant bacteria and/or resistance determinants contaminating food through the routes identified above, to identify the ones of highest priority for public health, if possible their relative importance, and the main risk factors influencing their occurrence and persistence in food-producing environments and food.

AQ2a. Among the antimicrobial-resistant bacteria and/or resistance genes contaminating food through the routes identified in this opinion, which are the ones of highest priority for public health?

- In food-producing environments, resistance to antimicrobials of choice for the treatment of serious bacterial infections or to last resort antibiotics was identified in bacterial pathogens (highest priority Group 1 bacteria) and in commensals or environmental bacteria encoded by mobile genetic elements (highest priority group 2 bacteria). Those ARGs usually associated with mobile genetic elements are considered of highest priority.
- Resistance to extended spectrum cephalosporins and fluoroquinolones was commonly reported. Carbapenems, colistin and glycopeptides resistance was also identified in bacteria/genes from different sources within plant-food and terrestrial animal food-producing environments. Resistance to oxazolidinones and plazomicin was rarely identified. For the aquaculture sector, reports of resistance to these antimicrobials were scarce or absent.
- Among the highest priority Group 1 bacteria, carbapenem/extended-spectrum cephalosporin/fluoroquinolone resistant MDR *Salmonella enterica*, extended spectrum cephalosporin resistant MDR Enterobacterales, fluoroquinolone-resistant *Campylobacter* spp., MRSA and VRE were identified**.**
- Highest priority Group 2 bacteria were frequently identified in several sources and sectors. MDR Enterobacterales (mostly *E. coli* and *K. pneumoniae*) resistant to extended-spectrum cephalosporins and/or fluoroquinolones were common. Extended-spectrum cephalosporin and colistin mobile resistance genes were also identified in *E. coli*. Of note, the identification of *Acinetobacter* spp. and MDR Enterobacterales with mobile resistance to carbapenems. Glycopeptide resistance in *E. faecium* or *E. faecalis*, as well as oxazolidinones resistant enterococci were also identified.
- Among the highest priority ARGs, those conferring resistance to carbapenems (e.g. bla_{VIM}, bla_{NDM}, $bla_{\text{OXA-48-like}}$, $bla_{\text{OXA-23-like}}$), extended-spectrum cephalosporins (e.g. $bla_{\text{CTX-M}}$, AmpC genes), plazomicin (*armA*), colistin (*mcr* genes), methicillin (*mecA, mecC*), glycopeptides (*vanA)* and oxazolidinones (*cfr*, *optrA*) have been reported from food production environments.
- Highest priority ARB and ARGs identified in the food production environments could originate from several sources, including manure, water, workers and wildlife at primary production and transport, lairage, slaughtering and meat processing at post-harvest level.
- Data to estimate the public health burden (e.g. in terms of DALYs) for antimicrobial resistant zoonotic and environmental bacteria is not available. Therefore, highest priority ARB was identified on food-producing environments based on the pathogenicity potential (e.g. based on species, serotype or sequence type) and resistance profile to last resort antimicrobials or presence in commensal or environmental bacteria of ARG conferring resistance to those antimicrobials in mobile genetic elements. The general relevance of this approach is supported by international documents and available research studies.
- Overall, ARB published data is often summarised and consolidated so that full granularity is not achievable, namely lack of reporting of MDR profiles and/or resistance mechanism of individual isolates or potential clinical relevance. The conclusions are supported by targeted literature screening conducted and availability of information on highest priority ABR/ARG within other settings linked with food sectors investigated.

AQ2b. Which are factors that make a considerable contribution to their occurrence and persistence in food-producing environments and food?

- Several general factors are identified, including selective pressure by different compounds (e.g. use of antimicrobials, heavy metals or biocides), introduction of ARB and ARGs via breeding pyramids, continuous cycling of bacteria between the animals and their environment, resulting from inadequate definition or implementation of biosecurity measures and, for post-harvest situations, food safety management systems (FSMSs) with ineffective food hygiene procedures, e.g. deficiencies in GMP/GHP/PRP/HACCP.
- Presence of bacterial traits linked to the general resilience (e.g. resistance to desiccation, temperature), biofilm formation, stress response, virulence, compensation of fitness costs and co-location of ARGs and/or genes conferring tolerance to heavy metals or biocides on the same genetic platform (e.g. plasmid) could enhance their occurrence and persistence in the food production environment.
- Replacement of animals and persistent environmental contamination are important factors involved in recontamination.
- The microbiota of natural environments, particularly soil, slow-moving water or sediment, is a natural reservoir and source of ARGs that could contribute to the occurrence and persistence of ARB/ARG in the food-producing environment.
- Overall, there are insufficient studies assessing several factors, e.g. minimum selective concentrations for different antimicrobials, heavy metals and biocides and on bacterial traits enabling persistence of ARB. However, there is evidence that the factors and bacterial traits identified in this opinion are important for ARB/ARG persistence.

ToR3: To review and, if possible, assess the impact of existing or new possible strategies and options to mitigate the risk of emergence, spread and food-borne transmission of the antimicrobial-resistant bacteria identified above.

AQ.3a. What are the possible strategies and options to mitigate the emergence and spread in the food-producing environment of the antimicrobial-resistant bacteria and resistance genes identified in this opinion?

- Apart from prudent AMU, the proper implementation of general measures focused on good hygiene practices and biosecurity to prevent and/or reduce the occurrence and transmission of animal and food-borne pathogens will mitigate ARB. These measures are the most important ones applicable for all the food-production sectors investigated, both at pre- and post-harvest, and are covered by EU Legislation and industry codes of best practice.
- Biological methodologies that focus specifically on the reduction/elimination of ARB in the food production sectors, such as CRISPR-Cas, phages or predatory bacteria are in the early phases of research and development in the AMR field. More data on the efficacy and potential risks of these systems are required before specific recommendations can be made on their utility.
- Activities at production stages which can widely disseminate large numbers of ARB and ARGs in the different production sectors are a priority for intervention. For all sectors, reducing faecal microbial contamination is a priority.

    - For the plant-based production sector, measures reducing the bacterial content of manure (e.g. by composting or anaerobic digestion), sewage sludge (e.g. by heat or chemical treatment such as liming) and irrigation water (see below) should be considered.
    - For terrestrial animals at the farm level, preventing transmission from other animals (e.g. by control of rodents, arthropods and wild birds), dust (e.g. by traps or filters), feed (e.g. heat or chemical treatment) or surface run-off water, is relevant. Cleaning and disinfection of equipment and surfaces, in particular to remove faecal material and bacteria, together with hygienic procedures for workers should be properly implemented.
    - For aquaculture systems, high microbial water quality should be assured by reducing contamination by human faecal waste (e.g. wastewater effluents) and run-off from terrestrial animal production systems. Reducing AMR contamination of fish feed should also be a focus of attention.
    - For post-harvest stages, the implementation of food safety management systems is currently the main mitigation and preventive strategy to minimise the risk

- Mitigations directed to prevent ARB and ARGs in different water sources (e.g. irrigation water, surface water and fresh and marine water of aquaculture) include:

  - Some advanced wastewater treatment technologies such as membrane filtration or reverse osmosis systems are effective approaches to remove ARB/ARGs in full-scale WWTPs.
  - Attention should also focus on reducing current raw sewage discharges and on improving conventional wastewater treatment (e.g. upgrading secondary treatment plants to tertiary treatment standards).
  - A multiple barrier approach to protect plant production and aquaculture, including low impact approaches (e.g. constructed wetlands), combined with improvements to existing and more advanced processes for urban wastewater treatment, is recommended.
- There are few studies on the efficiency of general mitigation options on ARB/ARG elimination. Available studies are supported on general bacterial contaminants.

AQ.3b What are the advantages and disadvantages of implementing these mitigation measures?

- More frequent use of biocides, or increasing their concentration, may control ARB more effectively, but could also lead to increased exposure of workers and greater environmental chemical pollution, and some biocides may co-select for AMR.
- Proper implementation of general measures focused on good hygiene practices, biosecurity and food safety management systems are also likely to provide better control of food-borne zoonotic pathogens, in addition to ARB, but may increase the use of water, biocides and energy.
- Biological methodologies may reduce persistent pathogens, but their ecological impact should be assessed.
- Improved treatment of faecal waste will reduce transmission of faecal pathogens but increases storage and equipment resources requirements and may reduce the fertiliser value.
- Preventing transmission from other animals (e.g. by control of rodents, arthropods and wild birds) will also reduce disease transmission but may also risk reducing the diversity of wildlife in rural areas and biocidal products such as rodent baits can also affect non-target species, particularly birds of prey.
- Improved heat treatment of feed will reduce the risk of ARB and pathogen transmission, but can reduce its nutritional quality, damage added vitamins and probiotics and utilise more energy.
- Improved treatment of wastewater will have a similarly beneficial effect on transmission of water-borne disease, and help to mitigate possible future water shortages due to climate change, but there are energy, resource and land use implications associated with the use of more advanced technologies and extensive constructed wetlands.

TOR4: To identify data gaps influencing the assessment of the food chain-related AMR risks posed by the environment and provide recommendations to inform future EU research priorities on this topic.

AQ4a. Which are the knowledge gaps influencing the assessment of the role played by the environment in the emergence and spread of antimicrobial resistance through the food chain?

- A large number of data gaps exist in relation to the relative importance of the multiple potential sources and transmission routes for ARB and ARGs in food-producing environments. The most detailed food production environmental studies have been carried out in situations where antimicrobial use or production methods and environmental protection laws, as specified by EU Legislation standards, do not apply.
- Knowledge on the diversity of ARB and ARGs/MGEs is limited by the lack of systematic studies using similar sampling frames and detection methodologies for ARB, ARGs and MGEs and the scarcity of studies that focus specifically on the production environment.
- Data on the effectiveness of some mitigation measures to specifically reduce ARB and ARGs is lacking. Therefore, despite a large number of studies that have investigated the occurrence of ARB and ARGs in food production, the role played by the environment is not sufficiently researched and there is insufficient data to support a specific assessment of the quantitative impact of contamination of the EU production environment on foods or public health.

AQ4b. Which future EU research priorities on this topic could be recommended?

- Comprehensive, integrated studies, linked to One Health initiatives and harmonised environmental AMR monitoring strategies by means of specific focal environmental monitoring or surveillance points are needed to establish the relevance of environmental sources for the introduction of AMR in food-producing systems. *Priority sectors would include plants-based food and aquaculture sector, due to the paucity of available studies.*

Within this topic area, the immediate priorities are to:

  - *optimise suitable sensitive and readily standardised culturomics/genomics-based detection methods for currently important and emerging ARB/ARGs.*
  - *define effective sampling strategies for different production environments that will facilitate a meaningful assessment of the occurrence, distribution and level of ARB/ARGs and, based on these studies, to identify efficient sampling frames, sampling points.*

- Long term longitudinal cohort studies on emerging and more widely established high priority ARB/ARGs, using advanced but standardised quantitative microbiology and genomic/ metagenomic based epidemiological methodologies in different representative countries within the EU, as well as studies involving environmental exposure of food animals in order to assess the biological relevance of different aspects of environmental contamination.
  - *Within this topic area, the most urgent priority is to focus on case studies on the occurrence and dissemination of emerging bacteria with highest priority ARGs (e.g. relating to carbapenems, tigecycline, isoxazolidinones, colistin) within food production environments and their dissemination into the wider/natural environment via waste, run-off, etc.).*
- Validating the efficacy of practical mitigation methods (e.g. current biosecurity and hygiene-based control programmes, environmentally friendly water treatment methods) would be also recommended.
  - *Immediate priorities within this work should be assessing and developing validated methods for disinfection/decontamination aimed at highest priority ARB in the production environment, heat treatment conditions for animal feed and treatment of faecal waste and wastewater used for fertilisation/irrigation and/or processing crops. Such studies would be expected to yield the most rapid benefits and could be linked with the epidemiological studies mentioned above.*
  - *Assessing the role and control of potential wildlife vectors.*
  - *Further development and validation of novel methods to control specific AMR dissemination in the environment*.
- These studies should be linked to assessment of the effect of future policy developments (e.g. within the EU Green Deal, Circular Economy and Veterinary Medicines products Regulation (EU) 2019/6) affecting food-producing environments, AMU, climate change impacts, maximising recycling of natural resources and the efficiency of food production for an expanding global population.

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Glossary

Terms used in the graphs:

In order to harmonise the terms used in the sector maps presented in 3.1, major terms grouping different related terms were used. Those terms are given below and are not to be considered as their strict definitions.

Animals

Companion animals: cats, dogs, non-food-producing horses, etc.
Arthropods: insects (e.g. flies, cockroaches, beetles), mites, etc.

Wildlife: mammals (e.g. wild boar, foxes), birds (including migratory), fish.
Rodents: mice and rats, etc.
Co-grazing animals: intended presence of more than one farmed animal species in outdoor food animal production.

Humans

Workers: farmers, farm staff, temporary workers, personnel from slaughterhouses, processing plants, fish processing, etc.
Visitors: external to the food-producing plants: veterinarians, auditors, farmer's family if not also working as farm staff, contractors, other persons from the community, etc.

Food-producing sectors/environments

Food: producing environments: all environments where food of animal or non-animal origin is produced or processed, at both primary level (e.g. plant-based foods, terrestrial animal farms, freshwater and coastal aquaculture farms, etc.) and post-harvest level (e.g. slaughterhouses, processing plants, etc.).
Plant-based food sector: production of vegetables, grains, fruit, nuts and other crops used for human or animal consumption, cultivated on fields, orchards, greenhouses, hydroponic production, associated processing plants.
Terrestrial animal sector: farming of poultry, cattle including dairy calves, veal and beef, pigs, slaughterhouses and processing plants.
Aquaculture sector: production of fish, crustaceans, molluscs, marine and freshwater farms and processing plants.

Food and Feeding stuffs

Food products: food products resulting from the different food-producing sectors intended for human consumption (meat, milk, eggs, vegetables/salads, fruits, fish, molluscs, crustaceans, etc.).
ABPs: animal by products (e.g. deadstock, waste eggs, abattoir and processing waste, etc.).
Feed: compound feed, home mixed feed, forages.
Pasture: grass or other grazing feedstuffs.

Chemicals

Antimicrobials: includes both active ingredients used for therapeutic and preventative medications, as well as the residues released.
Biocides: different chemical substances (e.g. detergents; alkaline or acidic degreasing agents) used to clean and disinfect animal housing, abattoirs and processing facilities and equipment, among other purposes. Also include preservatives used in food conservation.
Heavy metals: copper, zinc, silver; used in feed or water to enhance growth and minimise intestinal disorders, or used as biocides.

Water

Drinking water: water supplied to food animals via constructed drinking systems; including municipal water and water from private bore holes and wells.
Surface water: waterbodies such as rivers/streams, lakes and ponds, brackish waters and salt waters that can contain ground water, rainwater, melt water and wastewater treatment plant effluent.
Irrigation water: water used to irrigate crops or grassland or to dilute agricultural chemicals applied to these areas. Sources can include drinking water, surface water, ground water or reclaimed water (effluent from a wastewater treatment plant where wastewater it is subjected to primary, secondary and tertiary disinfection treatments, after which it can be reused for other purposes).
Process water: water that is used during the processing of food of animal and non-animal origin. Process water can consist of e.g. drinking water or source water and is used in different production and processing steps such as rinsing, washing, mixing, etc.
Run-off: water which flows from agricultural fields, pastures, animal farms or waste storage areas to surface water, e.g. after heavy rain.
Ice: Ice used for storage and/or transport of food (e.g. for aquaculture products).

Livestock and human faecal waste

Manure: faecal waste from livestock animals for fertilisation of agricultural land with nutrients, including slurry, pooled faecal material, faecal material incorporated in used bedding (e.g. poultry litter, farm yard manure), and treated manure (i.e. manure that has been treated by techniques such as composting, solid/liquid separation, anaerobic digestion or heat treatment).
Wastewater: water that is faecally contaminated by human waste (domestic wastewater, also known as sewage) or by animal faecal waste, such as in slaughterhouses. This includes both raw, untreated wastewater (released to surface water e.g. from combined sewer overflows) as well as effluent resulting from wastewater treatment.
Sewage sludge: treated solids recovered from the treatment of sewage. Sewage sludge can be used for soil improvement and nutrition on agricultural land.
Fertiliser: organic fertilisers of faecal origin, such as manure, and treated faecal material (products intended to provide plants with nutrients).

Others

Soil: the upper layer of earth in which plants grow, typically consisting of a mixture of organic remains, and mineral particles such as clay, and sand.
Growth substrate: this relates to substrates in the natural environment, such as residues of faecal, feed or food material, decomposition products from plant or animal material or microbiota.
Covered crops: Covered crops are plants grown under covers or in greenhouses.
Air: air in the context of this opinion relates to air that can move into, within and between food production premises, either as a result of natural circulation of air or ventilation systems.
Dust: fine particles of solid matter that can accumulate on surfaces or be carried in the air. When originating from animal farms: including dried faecal matter, dried soil, sloughed cells from the integument, feed or bedding material.
Bedding: plant-based material, such as wood shavings or straw which is used for food animals as a comfortable protective and warm, dry layer between them and floor surfaces.
Breeding lines: a specific genetic type of food animal, especially relating to poultry and pigs, which forms the basis of primary or nucleus breeding stock. These animals may be pure breeds, or hybrids derived from cross-breeding.

Abbreviations

AMEG	Antimicrobial Advice ad hoc Expert Group
AMU	Antimicrobial use
AMR	Antimicrobial resistance
ARB(s)	Antimicrobial-resistant bacteria(s)
ARG(s)	Antimicrobial resistance gene(s)
BIOHAZ	EFSA Panel on Biological Hazards
bla	beta-lactamase genes
BP	Before present
CAC	Codex Alimentarius Commission
CAC/PRP	Codex Alimentarius Commission Prerequisite Program
CA-MRSA	Community acquired MRSA (see below)
CC	Clonal complex
CDC	U.S. Centers for Disease Control and Prevention
CFU	Colony-forming unit
CIA(s)	Critically important antimicrobial(s)
Codex	Alimentarius International 'Food Code' established by FAO and WHO Organization to develop and harmonise international food standards.
CRE	Carbapenem-Resistant Enterobacterales
Cu	Copper
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
ESC	Extended spectrum cephalosporines
EEA	European Economic Area
EMA	European Medicines Agency
ESBLs	Extended-spectrum beta-lactamase(s)

EQS	Environmental Quality Standards
EUROSTAT	European Statistical Office
EUSR	European Union Summary Report
FAO	Food and Agriculture Organization
FCI	Food Chain Information
GE(s)	Genetic element(s)
GHP	Good Hygiene Practise
GMP	Good Manufacturing Practice
HA-MRSA	Hospital acquired MRSA (see below)
HACCP	Hazard analysis and critical points
HGT	Horizontal gene transfer
IACG	United Nations Interagency Coordination Group
ICE(s)	Integrating and conjugative element(s)
IS	Insertion sequence
JIACRA	Joint Interagency Antimicrobial Consumption and Resistance Analysis (EU)
JPIAMR	Joint Programming Initiative on AMR
MGE(s)	Mobile genetic element(s)
LA-MRSA	Livestock-associated MRSA (see below)
LMICs	Low middle income countries
MDR	Multidrug-resistant
MGE	Mobile genetic element
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS(s)	Member State(s)
MSC	Minimum selective concentration
NETESE	Network for Enhancing Tricycle ESBL Surveillance Efficiency
OECD	Organisation for Economic Co-operation and Development
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PiE	European Union Strategic Approach to Pharmaceuticals in the Environment
RAS	Recirculating aquaculture system
PS	Priority Substances
RCP	Recommended Code of Practice
RONAFA	EFSA-EMA Reduction of the Need for Antimicrobials in Food-producing Animals Scientific Opinion
spp.	Species (plural)
ST	Sequence type
SWOT	Strengths, weaknesses, opportunities, and threats
TET	Tetracycline
ToR(s)	Term(s) of Reference
UN	United Nations
UNEA	United Nations Environment Assembly
UNEP	United Nations Environment Programme
VBNC	Viable but nonculturable
VRE	Vancomycin-resistant enterococci
WASH	Water, Sanitation and Hygiene (WHO)
WG	Working group
WGS	Whole genome sequencing
WHO	World Health Organization
WL	Surface Water Watch List
WWT	Wastewater treatment
WWTP(s)	Wastewater treatment plant(s)
Zn	Zinc

Antimicrobials:

AMP	ampicillin
AMX	amoxicillin
AZT	aztreonam
CARBA	carbapenems
CHL	chloramphenicol
COL	colistin
ERY	erythromycin
FQ(s)	fluoroquinolone(s)
GEN	gentamicin
KAN	kanamycin
NEO	neomycin
SUL	sulfonamide
STR	streptomycin
TET	tetracycline
TMP	trimethoprim
VAN	vancomycin
3rd-GC(s)	third-generation cephalosporin(s)

Appendix A – Legal background

Relevant EU legislation related with topics addressed in this opinion and currently in force is listed below:

Monitoring of AMR in bacteria from food-producing animals and derived meat

- Regulation (EC) 178/2002[43] Article 33 establishes that EFSA shall search for, collect, collate, analyse and summarise relevant scientific and technical data in the fields within its mission. This shall involve, in particular, the collection of data relating to incidence and prevalence of biological risk.
- Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents lays down the provisions for monitoring of AMR in zoonotic agents and, in so far as they present a threat to public health, other agents at the stage or stages of the food chain most appropriate to the zoonosis or zoonotic agent concerned. The Directive requires EU MSs to collect relevant and, where applicable, comparable data on zoonoses, zoonotic agents and AMR, and to investigate food-borne outbreaks.
- Implementing Decision 2013/652/EU on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria was adopted as part of the 2011–2016 European Commission action plan. It applied from 2014 to 2020 and set up harmonised requirements for the monitoring of AMR in food-producing animals and food from a public health perspective. It was repealed and replaced by Commission implementing Decision (EU) 2020/1729 cited below.
- Commission implementing Decision (EU) 2020/1729 of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria. This Decision lays down harmonised rules for the period 2021–2027 for the monitoring and reporting of antimicrobial resistance ('AMR') to be carried out by Member States and is entered in into force from 1 January 2021 on.

Water

- Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy (OJ L 327, 22.12.2000, p. 1).[44] A fitness check evaluation of this Directive was published in 2019,[45] as a result of which, impact assessment being prepared for daughter directives on groundwater 2006/118/EC and environmental quality standards 2008/105/EC cited below.
- Council Directive 91/271/EEC of 21 May 1991 concerning **urban wastewater treatment** (OJ L 135, 30.5.199).[46] The Directive will be reviewed after performing an impact assessment; an evaluation of the Directive has been performed.[47]
- Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for **human consumption** (OJ L 330, 5.12.1998, p. 32).[48] It is repeal with effect from 13 January 2023 by the Directive (EU) 2020/2184 cited below.
- Directive (EU) 2020/2184 of the European parliament and of the council of 16 December 2020 on the quality of water intended for human consumption (recast).
- Regulation (EU) 2020/741 of the European parliament and of the council of 25 May 2020 on **minimum requirements for water reuse.**[49]
- Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of **bathing water quality** and repealing Directive 76/160/EEC (OJ L 64, 4.3.2006, p. 37).[50] Awaiting evaluation[51]

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[43] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[44] https://eur-lex.europa.eu/resource.html?uri=cellar:5c835afb-2ec6-4577-bdf8-756d3d694eeb.0004.02/DOC_1&format=PDF

[45] https://ec.europa.eu/environment/water/fitness_check_of_the_eu_water_legislation/documents/Water%20Fitness%20Check%20-%20SWD(2019)439%20-%20web.pdf

[46] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31991L0271&from=EN

[47] https://ec.europa.eu/environment/water/water-urbanwaste/evaluation/index_en.htm

[48] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31998L0083&from=EN

[49] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R0741&from=EN

[50] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006L0007&from=GA

[51] https://ec.europa.eu/environment/water/water-bathing/index_en.html

- Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of **groundwater** against pollution and deterioration (OJ L 372, 27.12.2006, p. 19).[52] Amended by 2014/80/EU cited below. Impact assessment being prepared (see WFD above).
- Commission Directive 2014/80/EU of 20 June 2014 amending Annex II to Directive 2006/118/EC of the European Parliament and of the Council on the protection of groundwater against pollution and deterioration.[53]
- Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources (OJ L 375, 31.12.1991, p. 1).[54]
- Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on **environmental quality standards** in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council (OJ L 348, 24.12.2008, p. 84).[55] Amended by Directive 2013/39/EU cited below. Impact assessment being prepared (see WFD above)
- Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.[56]

Sludge, manure, animal by products, other

- Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture (OJ L 181, 4.7.1986, p. 6).[57] It was evaluated in 2014, and specific aspects will be/are re-evaluated.[58]
- Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation) (OJ L 300, 14.11.2009, p. 1).[57]
- Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive (OJ L 54, 26.2.2011, p. 1).[59]

Food and feed hygiene and microbiological criteria for foodstuffs

- Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs (OJ L 139, 30.4.2004, p. 1.).[60]
- Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene (OJ L 35, 8.2.2005, p. 1.).[61]
- Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (OJ L 338, 22.12.2005, p. 1).[62] This regulation has suffered several amendments included in the consolidated version of 8/3/2020.[63]

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[52] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006L0118&from=EN

[53] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014L0080&from=EN

[54] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31991L0676&from=EN

[55] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/? https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0142&from=EN

[56] https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:EN:PDF

[57] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1069&from=EN

[58] https://ec.europa.eu/environment/waste/sludge/#:~:text=Evaluation%20of%20the%20Sewage%20Sludge,%2C%20vegetation%2C%20animals%20and%20humans

[59] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0142&from=EN

[60] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004R0852&from=EN

[61] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R0183&from=EN

[62] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R2073&from=EN

[63] https://eur-lex.europa.eu/eli/reg/2005/2073/oj/eng

Others:

- Also relevant are the European Green Deal initiatives such as the EC Farm to Fork strategy[64] and the Zero Pollution Action Plan (under development, due May 2021) and the EU Strategic Approach to Pharmaceuticals in the Environment.
- Regulation (EU) 2019/6 of the European parliament and of the council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/. It entries into force on 28 January 2022 (OJ L 4/43 7.1.2019).

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[64] https://ec.europa.eu/food/farm2fork_en

Appendix B – Other previous EFSA Opinions of interest to this Mandate

In 2008, the BIOHAZ Panel was asked to identify the extent to which food serves as a source for the acquisition, by humans, of ARB or bacteria-borne ARGs, to rank the identified risks and to identify potential control options for reducing exposure (EFSA, 2008a). At that time, the extent of exposure to ARB was found to be difficult to determine, and the role of food in the transfer of ARGs was insufficiently studied. Among the ARB involved in human disease that could be spread through food, resistant *Salmonella* (spread through poultry meat, eggs, pork and beef), and *Campylobacter* (poultry meat), played a major role. Other bacteria identified were AMR verocytotoxin producing *Escherichia coli* (through bovine meat), and potentially, MRSA (through animal-derived products), possibly via handling meat rather than consumption. Food was also considered an important source for human infections with antimicrobial resistant *Shigella* spp. and/or *Vibrio* spp.

Since then, EFSA has produced several reports and scientific opinions in which different aspects related to the public health risks of AMR, and specific ARB or ARGs in food and food-producing animals have been analysed.

In 2011, a Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum beta (β)-lactamases (ESBL) and/or AmpC β-lactamases (AmpC) in food and food-producing animals was delivered (EFSA BIOHAZ Panel, 2011a,b). Some aspects related to the food-producing environment were reviewed in this report. It was concluded that there was limited evidence for spread of ESBL/AmpC-carrying organisms via direct contact with food-producing animals or indirectly via the environment. Moreover, because the most evidence was available for high prevalence of ESBL/AmpC-producing bacteria in the poultry production pyramid, and their consequent possible involvement in public health, it was considered of highest priority, among other things, to prevent local recirculation within subsequent flocks. To reduce the public health risk caused by ESBL and/or AmpC-producing bacterial strains transmitted via the food chain or via food animal production environment, measures aimed at the control of dissemination (increased farm biosecurity, improved hygiene throughout the food chain), and other general post-harvest controls for food-borne pathogens were identified as relevant.

In 2013, a scientific opinion on carbapenem resistance in food animal ecosystems was delivered (EFSA BIOHAZ Panel, 2013a,b). The carbapenemase genes and their bacterial producers relevant for public health and linked to food-producing animals or food-borne transmission were defined. Some carbapenemase-producing isolates of *Salmonella enterica* and *E. coli* had been sporadically identified in the food-producing environment (pig and/or broiler farms). Nevertheless, after reviewing the information on the epidemiology of acquired resistance to carbapenems, it was concluded that the transmission of carbapenemase genes or their bacterial producers to humans through the food animal production environment or food chain was not reported at that time (2013), but it was considered likely should these strains/genes spread more widely in food-producing animals.

In 2017, EFSA and EMA delivered a scientific opinion on '*measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union (EU) and the resulting impacts on food safety, taking into account the impact on public health and animal health and welfare*' (EMA and EFSA, 2017). Overall, it was assumed that a reduction in AMU would result in a general reduction in AMR in bacteria from food-producing animals and food. Among the measures that were mentioned relevant for the present scientific opinion (release of ARB into the farm environment), it was considered that animal husbandry and disease prevention measures that could be implemented to improve animal health and welfare, and therefore reducing the need to use antimicrobials, should be implemented. Those measures, could be divided into three main categories, including practices to reduce the introduction and spread of microorganisms between farms (e.g. external biosecurity, compartmentalisation and eradication measures), to reduce transmission or spread within a farm (e.g. internal biosecurity, production groupings, housing design, building and maintenance), and to increase the ability of animals to cope with pathogens (e.g. housing, nutrition, stress reduction, vaccination and genetic selection). Measures which reduce the need to use antimicrobials, such as improved biosecurity, control and/or eradication of infectious diseases and the alternatives identified above, are likely to reduce the development of AMR indirectly. Some substances which are used as alternatives to antimicrobials (e.g. zinc oxide) may also increase selection pressure for AMR. To reduce AMU in the livestock industry an integrated, multifaceted approach should be taken. Some recommended options for reducing AMU and the need for antimicrobials, that could be relevant for the present scientific opinion were: establishing targets for reduction of AMU, especially critically important antibiotics (CIAs); development and use of on-farm animal health management with professional input; increased oversight of preventive and metaphylactic antimicrobial use; improvement of husbandry and management procedures for disease prevention, control and eradication in livestock production, including vaccination. Rethinking livestock production systems including reduced reliance on AMU and exploring further the potential of alternative production systems; and, development of treatments as alternatives to antimicrobial substances were also considered to be important (EMA and EFSA, 2017). Some of these measures have been incorporated into the new Regulation 2019/6 on Veterinary Medicinal Products.

In 2017, ECDC, EFSA and EMA delivered a joint scientific opinion on *a list of outcome indicators to assist EU MSs in assessing their progress in reducing the use of antimicrobials and AMR in both humans and food-producing animals* (ECDC, EFSA and EMA, 2017a). 1) For humans, the proposed indicators for AM consumption are: total consumption of antimicrobials (limited to antibacterials for systemic use), ratio of community consumption of certain classes of broad-spectrum to narrow-spectrum antimicrobials and consumption of selected broad-spectrum antimicrobials in healthcare settings. The proposed indicators for AMR are: MRSA and 3rd-generation cephalosporin-resistant *E. coli*, *K. pneumoniae* resistant to aminoglycosides, fluoroquinolones and 3rd-generation cephalosporins, *S. pneumoniae* resistant to penicillin and/or macrolides and *K. pneumoniae* resistant to carbapenems. 2) For food-producing animals, proposed indicators for AM consumption included: overall sales of veterinary antimicrobials, 3rd- and 4th-generation cephalosporins, quinolones and polymyxins. Indicators for AMR: full susceptibility to a predefined panel of antimicrobials in *E. coli*, proportion of samples containing ESBL-/AmpC-producing *E. coli*, resistance to three or more antimicrobial classes in *E. coli* and resistance to ciprofloxacin in *E. coli*. The chosen indicators should be reconsidered at least every 5 years. The indicators proposed provide a broad overview of the situation considering the main food-producing animal populations together and enable detecting beneficial impact of action plans against AMR covering the main production sectors concurrently.

In recent years, EFSA has revised and produced several *scientific opinions addressing the risk posed by different pathogens associated with foods of non-animal origin, FoNAO* (EFSA BIOHAZ Panel, 2011a,b, 2013a,b, 2014a–e; EFSA, 2011a–c). In these opinions, FoNAO general commodities categories were defined (e.g. different types of fruits, vegetable fruits, leaves, root and tuberous vegetables, others, EFSA BIOHAZ Panel, 2013a,b), and specifically evaluated (EFSA, 2011a,b; EFSA BIOHAZ Panel, 2014a–e). Descriptive analysis of the entire production processes for specific FoNAO were provided, indicating risk factors for contamination by specific pathogens. The contribution of the agricultural production environment, processing, distribution and retail/catering/domestic environments (subject to cutting, washing, peeling, shredding, freezing, mashing and unpasteurised juicing or blending) were considered. In EFSA BIOHAZ Panel (2013a,b) specific food/pathogens combinations most often linked to food-borne human cases originating from FoNAO in the EU were identified and ranked; the main risk factors for the specific food/pathogen combinations including agricultural production systems, origin and further processing, were identified; possible specific mitigating options were recommended and their effectiveness and efficiency to reduce the risk for humans posed by food/pathogen combinations was assessed. When relevant, microbiological criteria for the identified specific food/pathogen combinations throughout the production chain were recommended. Also, data gaps and research needs were identified, and recommendations were given. It was concluded that ‘microbial food safety hazards and sources of contamination may vary significantly by the type of crop, production systems and practices, and from one particular setting/context to another, even for the same crop’. In that opinion AMR was not considered in detail; however, it was concluded that consumption of food of non-animal origin may be of importance in the exposure of consumers to this hazard.

Appendix C – Literature searches performed for Tables presented in Section 3.2

In addition to the generic literature searches described in Section 2.2, additional targeted searches were performed to find European studies that could inform the review of ARB and ARG that contaminate the different food-producing environments through the main sources and transmission routes that were identified in Section 3.1.

	Common strings		Topic				Specific strings/number of hits[(a)] for each specific production sector									
							Plants-based		**Bovine**		**Pigs**		**Poultry**		**Aquaculture**	
PubMed (https://pubmed.ncbi.nlm.nih.gov/) (Status up to 01 March 2021)	(Germany OR Italy OR France OR UK OR Britain OR Netherlands OR Spain OR Sweden OR Norway OR finland OR Greece OR Portugal OR Belgium OR Poland OR hungary OR Ireland OR Scotland OR Luxembourg OR malta OR Latvia OR Lithuania OR Estonia OR Czech OR Croatia OR Austria OR Slovakia OR Slovenia OR Europe* OR EU)	AND	1) (ctx-m OR SHV OR ESBL OR 'extended spectrum' OR TEM)	AND	– – –	AND	(manure OR sludge OR irrigation)	192	(cattle OR dairy OR veal OR beef)	382	(pig OR swine OR sow OR wild boar)	344	(poultry OR chicken OR turkey)	615	(aquaculture OR fish OR shellfish OR bivalve* OR mussel* OR oyster* OR salmon OR shrimp* OR prawn*)	323
			2) (carbapenem OR carbapenemase)		a) – b) ('antimicrobial resistan*' OR 'antibiotic resistan*')		(manure OR sludge OR irrigation OR dust)	79		46		75		214		28
			3) ('Enterococcus faecium' OR '*Acinetobacter baumannii*' OR '*Pseudomonas aeruginosa*' OR '*Klebsiella pneumoniae*' OR '*Staphylococcus aureus*').		a) (mcr OR fluoroquinolone OR colistin OR 'antimicrobial resistan*' OR 'antibiotic resistan*')			419		345		360		554		194
					(mcr OR fluoroquinolone OR colistin OR 'antimicrobial resistan*' OR 'antibiotic resistan*')			67		55		62		152		22
			4) (salmonella OR campylobacter)					43		205		259		516		30
			5) *E. coli*					181		393		387		554		76
			(antibiotic)		(dust OR airborne)		(agriculture OR farm OR poultry OR swine OR slaughterhouse OR dairy)/56 hits								–	–

(a): When large number of hits were obtained, they were skimmed briefly for titles which lead to rejection of a fraction of hits, then, after screening the abstract and affiliation and only a small fraction from these was selected based on expected contents for full text reading. In several cases, only a handful from the publications screened were of interest. For Tables F.1 and F.2 (Appendix F), a hierarchical search strategy was used. In a first search, bacteria co-existing with various fish species (i.e. Atlantic salmon, rainbow trout, European whitefish, carp, sea bass, European eel) were identified. Then in a second search keywords were used to identify the resistances of the bacteria identified in the first search.

Appendix D – Uncertainty analysis

Table D.1: Qualitative evaluation of the influence of uncertainties on the conclusions of the current opinion

Sources of uncertainties	**Impact of the uncertainty on the conclusions**			
General	**AMR sources and transmission routes identification in the food-producing environments investigated**	**ARB and ARG of highest public health importance present in the food-producing environments investigated**	**Factors contributing to AMR occurrence and persistence in food-producing environments and food**	**Strategies and options to mitigate the emergence and spread in the food-producing environments investigated**
• Insufficient studies addressing systematically AMR load and diversity in environmental sources or AMR mitigation strategies in recent times and covering EU diverse regions.	It is expected that the impact of this uncertainty is relatively low because of the availability of studies on general bacterial contaminants, evidence of introduction of pathogens, presence of ABR/AGR for all sectors and most sources and due to the experience of the working group members and the BIOHAZ panel members.	It is expected that the impact of this uncertainty is relatively low for all sectors, except for aquaculture (moderate), because of the availability of studies within other settings (e.g. human clinical and community settings, wider environment, wild animals and companion animals) showing the circulation of the ABR/ARG considered of highest priority in this SO in food animal production.	NA	It is expected that the impact of this uncertainty is relatively low because of the availability of studies on general bacterial contaminants and due to the experience of the working group members and the BIOHAZ panel members.
• Detection sensitivity of test methods (e.g. some culturomic and metagenomic) for AMR threats in environmental samples is largely unknown, or known to be insensitive.	It is expected that the impact of this uncertainty is relatively low due to better sensitivity of the enrichment methods for specific detection of most relevant ARB/ARG and bacterial contaminants, and due to the experience of the working group and the BIOHAZ panel members.	The expected impact is moderate to high regarding more recently recognised AMR threats (e.g. carbapenems, oxazolidinones or glycylcyclines resistance genes) or particular organisms (e.g. *Acinetobacter* spp. or *Klebsiella* spp.	It is expected that the impact of these uncertainties is relatively low due to availability of representative works with bacterial contaminants and the experience of the working group members and the BIOHAZ panel members.	It is expected that the impact of these uncertainties is relatively low due to availability of representative works with bacterial contaminants and the experience of the working group members and the BIOHAZ panel members.
• Frequently no data on the potential clinical relevance, epidemicity (sequence type (ST), plasmid Inc type), persistence traits, of ARB, ARGs or MGEs from environmental samples. Limitations in the	A low impact of the uncertainties related to these factors is expected. More studies are required in which plants/animals are exposed to sources with different ARB/ARG carried by diverse MGEs and studied by	A relatively low impact of the uncertainties related to these factors on ABR/ARG considered of highest priority is expected, because of availability of studies supporting their relevance and due to the experience of the working	It is expected that the impact of these uncertainties is relatively low, to, as there is sufficient evidence that the traits identified are relevant. More studies are required to provide further insights on other possible traits enabling ARB persistence and the ability to	A low impact of the uncertainty related to this factor on ARB mitigation strategies is expected (please see previous cell), but a higher impact in relation to ARG mitigation strategies.

Sources of uncertainties	Impact of the uncertainty on the conclusions			
General	**AMR sources and transmission routes identification in the food-producing environments investigated**	**ARB and ARG of highest public health importance present in the food-producing environments investigated**	**Factors contributing to AMR occurrence and persistence in food-producing environments and food**	**Strategies and options to mitigate the emergence and spread in the food-producing environments investigated**
pathogenicity potential, epidemicity prediction based on ST, plasmid Inc type and co-location of ARG or heavy metals/ biocides in the same genetic platform, and HGT.	quantitative microbiology and metagenomics.	group members and the BIOHAZ panel members.	compete with other organisms. The drivers and the extension of ARG transfer between ARB present in the food environments is poorly known.	
• The public health burden (e.g. in terms of DALYs) of antimicrobial resistant zoonotic and environmental bacteria is undefined. Similarly, no robust data is available on the significance of ARG/ MGE transfer between bacteria in food and the human intestinal microbiome, or the significance of such events in terms of human disease.	NA	It is expected a moderate impact of these uncertainties in the ARB/ ARG identified as of highest relevance would apply, based on current medical knowledge of major sources of infectious diseases. More studies are needed, namely to provide data on number and severity of human diseases caused by the list of highest priority antibiotic-resistant pathogens as well as the fraction of those infections that are of food-borne origin.	NA	NA

Note: Regarding the level of the impact of the uncertainties, the terms used were ranked in the following order: low< relatively low< moderate.

Appendix E – Sources and transmission routes, additional information for terrestrial animals and aquaculture production

E.1. Terrestrial animal production sector

E.1.1. Poultry production sector

Poultry production is one of the most rapidly expanding global industries, with the total bird population expected to reach 8.5 billion by 2030 (DeSa, 2015). The European Union (EU) produced 13.3 million tonnes of poultry meat in 2019, which is an increase of about 27% since 2010. In 2019, poultry meat production was largest in Poland, Spain, France, Germany and Italy (Eurostat).[65,66]

ARB, including human-pathogenic strains from poultry, are regularly found in meat (EFSA BIOHAZ Panel, 2019b; EFSA and ECDC, 2021a), especially from poultry meat exporting regions where control of antimicrobials is less developed (Koga et al., 2015; Campos Calero et al., 2018; Mąka et al., 2018; Rozman et al., 2018; Tansawai et al., 2018; Rabello et al., 2020; Xu et al., 2020). Antimicrobials were commonly used in the EU, prior to Regulation (EC) No 1091/2005[67], for suppression of *Salmonella* to avoid detection of infection in the breeding pyramid and in meat birds prior to slaughter. Such routine use selected for resistant clones of *Salmonella* and other bacteria such as fluoroquinolone resistant *E. coli* and *Campylobacter* which are still circulating in the poultry industry, despite dramatically reduced usage in most EU countries (Edel, 1994; Reynolds et al., 1997; EFSA, 2004; Mouttotou et al., 2017; Kadykalo et al., 2019; Perrin-Guyomard et al., 2020).

Strategic preventive or therapeutic medication in breeding chicken flocks or of eggs or chicks in hatcheries to prevent avian disease was also a common occurrence prior to restrictions in 2012. This has created a large and important source of ongoing infection and contamination of birds and poultry meat with AMR pathogens and commensal organisms of the intestine and integument, especially regarding plasmid-mediated resistance to extended spectrum cephalosporins after routine use of ceftiofur in hatcheries (Seo et al., 2020).

Numerous studies have demonstrated that antimicrobial resistant zoonotic pathogens and commensal bacteria can be widely disseminated via the poultry breeding, rearing and production pyramid, both in response to current selection by medication and long-term persistence in the farm and hatchery environment as a result of suboptimal cleaning and disinfection, which can also select for AMR in surviving bacteria (Nhung et al., 2016).

The main factors influencing AMR presence in poultry (also see Table 2) relate to the production pyramid environment and historic or current antimicrobial use. With respect to the role of the production pyramid, clonal expansion of *E. coli* with resistance to 3rd-generation cephalosporins has occurred in several Nordic countries, despite the lack of usage for poultry in those countries, and is suspected to be associated with international trade in breeding stock and ongoing contamination of the environment within breeding farms, breeder rearing farms and hatcheries (Mo et al., 2014; Myrenås et al., 2018). A European Union study of four broiler parent flocks identified closely related PFGE subtypes of avian-pathogenic *E. coli* (APEC), which are normally resistant to multiple antimicrobials and result in reduced performance and disease (van Limbergen et al., 2020), in parent birds, newly hatched chicks and one-week-old chicks suffering mortality (European Commission, 2019). In laying hens, ARB introduced with replacement birds reduce during the laying period as birds become older and are rarely medicated, but resistant organisms still persist at a low level between flocks (Koyama et al., 2020). Overall, the intensity and pattern of antimicrobial use is the major driver for very complex patterns of resistance and associated metagenomes on poultry farms (Pesciaroli et al., 2018; Xiong et al., 2018) but ARB, although often reduced, are still common in organic poultry farms (Musa et al., 2020). Aspects of biosecurity and farm hygiene have also repeatedly been identified as factors responsible for the introduction and environmental persistence and cycling of flock

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[65] Editorial note included in the Eurostat reference cited: 'Throughout this article, which deals with time periods when the United Kingdom was a Member State of the European Union, the acronym EU, however, refers to EU-27, the post-Brexit composition of the European Union as of 1 February 2020'.

[66] https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agricultural_production_-_livestock_and_meat&oldid=470510#Poultry (last accessed February 2021).

[67] Commission Regulation (EC) No 1091/2005 of 12 July 2005 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards requirements for the use of specific control methods in the framework of the national programmes for the control of salmonella. OJ L 182, 13.7.2005, p. 3–4.

infections of AMR on farm premises, and there are large gaps in our understanding of the most important risk factors and the most effective interventions (Davies and Wales, 2019).

Detailed information on those sources common to all animals is included in Section 3.1.2, additional information is presented below.

E.1.1.1. Poultry preharvest AMR sources and transmission routes

Co-grazing animals

Free range poultry farms often use co-grazing with sheep or horses as a means of grassland management or may occasionally use camelids as a deterrent for predation by foxes.

One study using (cgMLST) schemes consisting of 1,140 and 529 genes for *C. jejuni* and *C. coli*, respectively, showed that nearby cattle, contaminated drinking water, water ponds, transport crates, and broiler parent flocks were potential reservoirs of *Campylobacter* for commercial broilers (Frosth et al., 2020), confirming earlier findings regarding cattle as a potential source (Bull et al., 2006). However, in this study, AMR is not mentioned.

Poultry farm waste

Specifically for poultry, external environmental contamination around poultry farms after poor cleaning and disinfection can occur (Battersby et al., 2017). From the apron area over which waste is incompletely removed after flock harvest, from the immediate house surrounds, stored litter or waste from other animals, reinfection with *Campylobacter* that carry varying AMR determinants can occur (Graham et al., 2009), including via movement of restocking-related vehicles into the house and return of litter beetles (Crippen and Poole et al., 2012).

E.1.1.2. Poultry post-harvest AMR sources and transmission routes

Much of the poultry meat that is used for further processing in many European countries is imported from third countries[68] where problems with widespread emergence of AmpC resistance, e.g. in *Salmonella* Heidelberg, are increasing. This presents a threat of introduction of this vertically transmitted organism into the EU poultry production environment via escape of organisms from processing waste or from infected humans, or pets consuming raw meat-based foods (Souza et al., 2020; Dazio et al., 2021).

Equipment

Broiler chickens carry ARB internally and on the integument at slaughter age even when no antimicrobials have been administered (Montoro-Dasi et al., 2020). Cross-contamination of poultry in transport crates and at slaughter contributes to greater microbial diversity in retail chicken than in live birds (Davis et al., 2011; Althaus et al., 2017). The scalding, defeathering and evisceration stages are the main sources of carcass contamination and release of micro-organisms into the slaughter environment, but all the high throughput automated or semi-automated processes are capable of disseminating contamination and there is no stage of the process that can significantly reduce contamination of carcasses, although more advanced slaughter equipment such as multistage counter-current scalding and rapid air chilling systems can help to reduce levels of bacterial contamination and indirectly AMR transmission (Rasschaert et al., 2020).

Poultry transport vehicles, modules and crates can also spread pathogen contamination between the abattoir and farms and between farms, particularly where partial sequential depopulation (thinning) is practiced (Buess et al., 2019; Rasschaert et al., 2020).

Abattoir and hatchery waste and wastewater

Rendered poultry abattoir and hatchery waste, as well as low grade category 3 abattoir waste, can be used for manufacture of feed for pets (including raw meat pet food) and farmed fish. Occurrence of AMR in such products is a means of further dissemination of AMR beyond the food chain (Hofacre et al., 2001; Groat et al., 2016; Davies et al., 2019).

Contamination and cross-contamination of workers, equipment and air can affect the further processing stages for chicken carcasses, with cleaning and disinfection procedures often being insufficient to eliminate bacteria contamination between working days, as shown for pathogens

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[68] https://ec.europa.eu/info/food-farming-fisheries/animals-and-animal-products/animal-products/poultry_en (last accessed February 2021).

(Samapundo et al., 2019; Obe et al., 2020). Feathers may also act as a means of introducing antimicrobial contamination into the poultry slaughter process, despite the approved withdrawal period for medications being observed (Cornejo et al., 2018). Eggshells were reported as a source for occupational exposure to airborne bacteria in turkey hatcheries (Brauner et al., 2016).

Process water and wastewater resulting from the slaughtering process can also be a source of contamination (Savin et al., 2020).

E.1.2. Cattle production sector

Cattle production, including both dairy cattle and cattle raised for production of veal and beef are major production lines in Europe as well as third countries providing a large variety of food derivatives for consumption in the EU and eventual exportation. In 2019 there were 77 million bovine animals registered in the EU, which were mostly concentrated in a few countries (France, Germany, Ireland, Spain, Italy and Poland). In 2019, the production of raw milk was 158.2 million tonnes (Eurostat)[69,70] which is mostly of bovine origin and used to produce cheese, butter and a variety of dairy products. The production of beef and veal was 6.9 million tonnes in the same year (Eurostat).[71]

Given the particularities of the species, of the existing breeds and of the production types, the factors influencing the spread of AMR will include sources and factors common to other terrestrial animals but also more specific ones which will be further described. Within the cattle production systems environmental sources might exert different degrees of influence depending on the production systems. E.g. systems with outdoor access and grazing are more likely influenced by external environmental sources of ARB and ARGs related to grazing than those where animals are kept indoors.

Environmental sources may contribute to dissemination to and circulation between and within farms, therefore maintaining a transmission cycle, as shown in Figures 9 and 10. Associated factors related to production systems might play a variable role in this dissemination, e.g. farm size and number of trade contacts or mixed farming units.

Dairy cattle and calf production

Dairy production relies on the farming of cattle for milk and dairy products, and dairy cattle and calves also contribute a significantly to meat production. Specific management practices associated with milk production are relevant for bacterial colonisation of the udder and therefore may act as sources for colonisation with resistant strains. Improper hygienic measures and/or barriers might increase the need for antimicrobial therapy and selection. E.g. for MRSA, a number of risk factors associated with the farm management and environment have been described such as: trade/acquisition of animals, improper milking hygiene, human carriage, contact with pigs, and others such as production system and herd size (Schnitt and Tenhagen, 2019; Dantas Palmeira and Ferreira, 2020).

Beef production

Beef production consists of rearing of beef from adult or near-adult animals as well as from calves. Calves can originate from within beef herds or from dairy cows mated to maintain the lactation cycle. For calves, a major risk factor for the introduction of AMR is the mixing of animals from different farms in one farm operation for rearing, along with suboptimal management and medication practices (Springer et al., 2019).

Detailed information on those sources common to all animals is included in Section 3.1.2. Additional information is shown below.

E.1.2.1. Cattle preharvest AMR sources and transmission routes

Bedding/soil

Bedding material has been found to harbour multidrug-resistant *E. coli* with similar resistance patterns as those observed in the calves sampled in the respective farms (Astorga et al., 2019).

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[69] Editorial note included in the Eurostat references cited: 'Throughout this article, which deals with time periods when the United Kingdom was a Member State of the European Union, the acronym EU, however, refers to EU-27, the post-Brexit composition of the European Union as of 1 February 2020'.

[70] https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Milk_and_milk_product_statistics&oldid=422287 (last accessed February 2021).

[71] https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agricultural_production_-_livestock_and_meat&oldid=470510#Veal_and_beef (last accessed February 2021).

Furthermore, it has been observed that calves can acquire cephalosporin resistant *E. coli* from bedding materials and that the urine of calves treated with cephalosporins may increase the selection of cephalosporin resistant *E. coli* in soils (Subbiah et al., 2012)

There is a risk of persistently contaminating the soil with ARGs in cattle facilities, which might vary for the different housing areas (Agga et al., 2019).

Milk/feed and feedstuffs

For dairy calves in the first stage, milk is the main nutrient and therefore a main source for colonisation with ARB. E.g. milk from cows treated during the lactating period is likely to contain a substantial amount of residues and would increase faecal shedding of ARB by calves (EFSA BIOHAZ Panel, 2017).

Waste milk is milk that cannot be marketed for human consumption, and this includes milk from cows treated with antimicrobials and also milk from cows that cannot be marketed for other reasons. Its use as feed may occur in different groups of calves that will not join the dairy herd i.e. being sold for meat production purposes (veal or beef), being raised as replacement stock for dairy animals or being kept on the farm for the production of veal or beef (EFSA BIOHAZ Panel, 2017). Waste milk containing high levels of ARB due to mastitis in cows is likely to contribute to transmission to calves as has been observed for ESBL producing bacteria in cattle farms in the UK (Horton et al., 2015). Discarded milk due to mastitis and antimicrobial treatment is shown to select for AMR in faecal flora of calves (Springer et al., 2019). This was also found to be the case for feeding pasteurised waste milk (Maynou et al., 2017).

E.1.2.2. Cattle post-harvest AMR sources and transmission routes

In milk and dairy production, milk is normally collected for processing and only a small percentage is locally processed at the farm. Bacteria from the skin and intestinal flora or other environmental sources at the farm (e.g. from bedding or other sources) might be sources of contamination of milk through contamination of the udder that can also result in environmental mastitis (clinical or subclinical). The pasteurisation process is quite effective at eliminating pathogens and thereby also ARB. However, there are possibilities of transmission to the food chain of ARB, also ARG, originating from the environment and the farm animals through products consumed raw, such as raw milk and artisanal cheeses prepared from raw milk (Alexa et al., 2020).

Culture based studies have shown that raw milk and dairy products based on raw milk carry potential pathogens with ARGs such as staphylococci, *E. coli*, *Campylobacter*, *Salmonella, Listeria* and others. The contribution of the environment for such contaminants is difficult to establish as similar environmental sources might be causing contamination both at farm and processing stages and some of the pathogens can originate from the animals (Costanzo et al., 2020).

For staphylococci, and particularly MRSA, host specificity might be associated with some specific lineages; however, it is known that MRSA are likely to be dispersed in the environment as well as persisting in biofilms on surfaces and within equipment in the dairy industry. This is especially critical if contamination occurs during the final processing stages where products will not be subjected to further processing that would reduce contamination (Papadopoulos et al., 2019).

E.1.3. Pig production sector

Pig production, with 22.8 million tonnes pig meat produced in 2019 (Eurostat),[72] is among the largest agriculture sectors within the EU. The EU is second only to China in terms of production. Current pig production is based mainly on integrated pyramids of production which can begin in feed milling and end at the slaughtering facilities. Most EU production is performed on confined industrial farms, while organic farming and extensive outdoor production are, so far, minor compared to conventional pig production figures.

Pig production is foremost in antimicrobial consumption within the EU (EFSA, 2017). Antimicrobials have been used to treat or control infectious pathologies intensified by the negative impact of conventional production on pig health. The following sections summarise the information gathered on evidence about environmental AMR sources for the pig production chain.

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[72] https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agricultural_production_-_livestock_and_meat&oldid=470510#Meat_production (last accessed February 2021).

Detailed information on those sources common to all animals is included in Section 3.1.2. Additional information is shown below.

E.1.3.1. Pig preharvest AMR sources and transmission routes

Farrow to finish versus multisite production systems

Pig production can be classified into two main production systems, although many combinations can co-exist. The first system is farrow-to-finish production, where sows, weaned pigs and fatteners are located in a unique herd within separate compartments, and the second system consists of multi-site production systems with specialised breeding herds located on different sites to nursery and finishing farms. The type of production will influence the impact of environmental effects among production stages. This is theoretically more plausible in farrow to finish herds, where transmission from environmental sources may occur between stages located within the same premises, but nursery and finishing farms may receive pigs from several different sources, which increases the risk of introducing undesirable organisms. However, we lack information on environmental presence and persistence of ARB in different herd types or production stages within a farm. In this regard, herds for breeding and selection purposes supply animals to production farms. These herds have high sanitary conditions, are specific pathogen-free and probably the use of antimicrobials is reduced compared to production herds.

Outdoor production

Outdoor pig farming is an alternative to conventional confined pig production (Mencía-Ares et al., 2020). Outdoor production systems include organic production or extensive free-range pig breeding and production. Outdoor farming is linked to a lower antimicrobial consumption and lower prevalence of ARB and ARGs (Österberg et al., 2006; Kempf et al., 2017; Mencía-Ares et al., 2020). Despite the evident relevance of the environment, most of the current information relies on studies focused on faeces or intestinal contents. A study of Novais et al. (2013) detected VRE in soil from outdoor reared pigs on a Portuguese farm. The role of persistence of bacteria in the soil was demonstrated by a study in UK in which moving outdoor pig herds to fresh land was associated with a reduction in MDR *S*. Typhimurium (Smith et al., 2017). Overall, more data is needed to establish the impact of the environment in these farming systems (Woolhouse et al., 2015).

E.1.3.2. Pig post-harvest AMR sources and transmission routes

Transport

Transport could be a contamination hotspot as trucks visit different farms, sometimes mixing animals from different origins. Studies on *Salmonella* and *Campylobacter* have demonstrated the presence of resistant strains inside trucks (Gebreyes et al., 2004; Quintana-Hayashi and Thakur, 2012), even in samples collected before loading the animals, presumably linked to inefficiency of cleaning protocols (Mannion et al., 2007).

E.1.3.2.1. Slaughterhouse

Lairage

Similar to transport, the resting area or lairage may be a source of AMR for pigs entering the slaughterhouse. Different batches of pigs are housed within the same facilities which may come in contact with ARB present in the environment (Rule et al., 2008; Walia et al., 2017). The burden of pathogens in the holding pens is relatively high and common, thus, there is a risk of introducing ARB into the slaughterhouse environment on contaminated skin and in the intestines.

Slaughter line

The slaughter line can spread AMR to carcasses. Contamination of water, machinery and workers' hands and their tools may introduce/spread ARB on the line (Gomes-Neves et al., 2012). Some points, principally scalding and singeing, could be potential critical control points (CCP) to reduce the burden of ARB (Wu et al., 2009; Van Gompel et al., 2020). Other points such as carcass polishing and splitting can result in cross-contamination between slaughter batches (Gomes-Neves et al., 2012; Melero et al., 2012). The air and aerosols from slaughter facilities may also favour the spread of AMR. A recent study has detected around 30% of air samples positive for *tet*W or *emr*B genes (Van Gompel et al., 2020). In contrast, other studies state that the transmission through air in slaughter facilities could be negligible (Pearce et al., 2006; Okraszewska-Lasica et al., 2014) although another study has demonstrated that filtered air reduces carcass contamination, but also highlights that other factors (such as carcass handling or processing) have more impact in bacterial surface contamination (Burfoot et al., 2006). Occupational transmission of AMR may occur at slaughterhouses (Mulders et al., 2010; van Cleef et al., 2010; Gilbert et al., 2012; Van Gompel et al., 2020) and at the same time workers, through their hands or equipment, may act as sources of AMR (Van Gompel et al., 2020). Resistome studies reveal variations in ARGs along the slaughter line and further emphasise the importance of good hygiene in carcass processing (Campos Calero et al., 2018; Van Gompel et al., 2020).

E.1.3.2.2. Processing plants

Carcasses are usually processed in cutting/processing plants where pork is processed to be delivered to retail establishments. In contrast to the abundance of information in AMR on pork carcasses, studies performed in processing plants, and particularly in the processing plant environment, are scarce. It can be assumed that meat introduced in the processing facilities acts as primary source of AMR (Argüello et al., 2013a), which then can be indirectly spread in meat processing. Despite the presence of food-borne pathogens, which is well documented (Giovannacci et al., 2001; Argüello et al., 2013b), the carriage of AMR is poorly investigated. Sala et al. (2016) conducted a study in a Romanian processing plant, where they evaluated the presence of *L. monocytogenes*. For food contact surfaces, 23.3% (14 of 60 samples) were contaminated with *L. monocytogenes* resistant to a number of antimicrobials, and among the analysed categories the pathogen was recovered from conveyor belts (5 of 15 samples; 33.3%), cutting surfaces (1 of 5 samples; 20%), packing surfaces (1 of 3 samples; 33.3%), personnel equipment (1 of 7 samples; 14.3%), processing equipment (3 of 11 samples; 27.3%), and slaughter equipment (3 of 12 samples; 25%). No significant differences in the distribution of the pathogen were found between food contact and non-food contact surfaces.

E.2. Aquaculture production sector

In 2018, the EU total production of fishery products was estimated to be 5.7 million tonnes live weight. Of this, ~ 1.1 million tonnes were accounted for by aquaculture production, with Spain, France, Italy and Greece as Member States with the largest production.[30,34] With 1.4 million tonnes, Norway is the leading aquaculture producer in Europe. In terms of output, Norway's aquaculture sector is the seventh largest worldwide and the EU is the world's ninth largest producer of aquatic organisms in 2018. Production covers a wide range of production systems and species, including coastal finfish production, small scale land based saltwater production systems, coastal and estuarine bivalve production which does not generally include feed or disease treatment, small scale crustacean production, marine algae (seaweed) production and freshwater finfish production which may be in rivers, lakes or tank systems. This diversity of production systems, in different environments and subject to a wide range of pollution sources means that aquaculture production has the potential to be contaminated by significant bacterial and chemical pollution.

Freshwater species are cultivated either extensively in ponds (particularly carp, often mixed with other species like zander and whitefish) or intensively in tanks (rainbow trout, eel, catfish and sturgeon). Ponds are similar to natural ecosystems with low organism densities and mainly natural feed. There are two different techniques of tank production for intensive farming: continuous flow systems and recirculation systems where water is nearly fully recycled and remains in the tanks. Recirculation aquaculture systems (RAS) are cost-intensive due to high energy usage but offer regulation of breeding conditions. RAS can also be used for cultivation of marine species. Marine species are reared either in shore-based tanks in a controlled environment with recirculating seawater (e.g. turbot, gilthead sea bream, sole) or in cages in sheltered zones near shore (particularly Atlantic salmon, sea bream, meagre). Species living in brackish water conditions (particularly eel, sea perch, sole, sea bream, sturgeon, shrimp and shellfish) are reared in lagoons, which also support conservation in coastal areas. For shellfish cultivation, different techniques such as ropes, wooden posts, tables or bottom-farming are used. Open systems, such as continuous flow systems or cages, can cause environmental damage when discharges containing antimicrobial residues or feed/faeces are released into the environment (Muziasari et al., 2017). The most commonly cultivated species is

Atlantic salmon which represents half of all the production in European aquaculture. In general, a high specialisation in aquaculture production at country level within Europe is observed (Eurostat).[73] An exception is the farming of rainbow trout which was cultivated in 34 countries in 2018 and represents nearly one tenth of total production. Other important cultivated species, are, Atlantic bluefin tuna (ranched, wild caught fish are fattened before slaughter), turbot, meagre, North African catfish, arctic char, European eel and other carp species as silver carp and bighead carp (FAO, 2020).

The most common use of antimicrobials is either treatment of individual fish (injection), adding the antimicrobial to feed (oral) or adding them directly to the water (bath). Injections are only used to treat large fish or highly valuable species whereas medicated baths are employed mostly for juveniles or larvae. There has been significant discussion of antimicrobial used in finfish aquaculture in Europe, with claims that due to low usage the risk of development of AMR is negligible (Lillehaug et al., 2018). However, as usage concentrations in aquaculture are several orders of magnitude higher than concentrations of environmental residues derived from livestock and human excretion, the relatively small amounts used (212 kg in Norway in 2016) may have increased significance in terms of driving selection for AMR in aquatic environments including aquaculture production systems (Bailey and Eggereide, 2020).

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[73] https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Fishery_statistics

Appendix F – Antimicrobial-resistant bacteria and resistance genes of high public health relevance in environmental sources of food-producing sectors, additional information

A detailed description of some relevant findings on public health highly relevant bacteria and genes presented in Section 3.2 and included in Tables 6–11 is presented below.

F.1. Plant-based food production sector

Summarised information on public health-relevant ARBs and ARGs identified in the plant-based sector is presented in Table 7 (Section 3.2.3.1).

A number of studies have revealed ARB in products at retail illustrating that Europeans are exposed to bacteria and ARGs of concern through consumption of fresh produce. A generally low prevalence of colistin resistant *E. coli*, colistin- and carbapenem-resistant *Pseudomonas aeruginosa,* linezolid resistant enterococci and staphylococci, and vancomycin-resistant enterococci have been reported (Reuland et al., 2014; Hölzel et al., 2018; Iseppi et al., 2018; Kaesbohrer et al., 2019; Manageiro et al., 2020).

Varying prevalence of ESBL-producing Enterobacterales was reported in different studies (Ruimy et al., 2010; van Hoek et al., 2015; Iseppi et al., 2018), with higher prevalence in an Italian study of various vegetables and ready to eat salads, or in a Dutch study of bean sprouts (Huizinga et al., 2018; Iseppi et al., 2018). Moreover, outbreaks of ESBL verotoxigenic *E. coli* or salmonellosis through consumption of contaminated fresh or processed produce are well documented (Bielaszewska et al., 2011; King et al., 2012; Colombe et al., 2019; Jechalke et al., 2019). AMR in *E. coli* was also reported in a Portuguese study in 17% of ready to eat salads with isolates resistant to tetracyclines, and lower frequency of resistance to streptomycin, sulfamethoxazole, trimethoprim, ampicillin, quinolones or chloramphenicol (15% to 8%) (Campos et al., 2013). A high prevalence of *Acinetobacter* spp. resistant to several antimicrobials, including ceftazidime, ciprofloxacin and imipenem, was reported from ready to eat fruits and lettuce collected in Portugal (Carvalheira et al., 2017).

A number of studies have revealed that phenotypically resistant bacteria obtained from produce at retail carry resistance determinants that are clinically relevant, and often on mobile genetic elements (e.g. $bla_{\text{SHV-12}}$, $bla_{\text{SHV-27}}$, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-27}}$, $bla_{\text{ACC-1}}$, $bla_{\text{DHA-1}}$, $bla_{\text{VIM-1}}$ and $bla_{\text{IMP-1}}$ encoding ESBLs and MBLs) (Huizinga et al., 2018; Iseppi et al., 2018; Kaesbohrer et al., 2019). Noteworthy is the presence of bacteria of clinical interest, e.g. *E. coli*-D-ST69 carrying $bla_{\text{SHV-2}}$ in ready to eat salads (Campos et al., 2013). The ability to transfer ESBL/AmpC and metalo-beta-lactamase encoding genes by conjugation at high frequency was associated with plasmids mostly carrying IncHI1, FIA and I1 replicons (Iseppi et al., 2018) or IncFIA, IncFIB, IncFII, Col and IncR (Huizinga et al., 2018).

F.1.1. Primary production environment

There are few studies concerning the abundance and characteristics of ARB in European plant-based food production environments.

In open plant production systems there are many potential opportunities for environmental contamination, as presented in Section 3.1. Some controlled studies have indicated the potential for AMR gene contamination in produce that is fertilised with animal manures (Marti et al., 2013). However, due to the absence of AMR monitoring, it is not possible to deduce where in the production and processing chain any particular food item at retail became contaminated.

Among the environmental sources, manure and irrigation water have been most studied; noteworthy is the presence of different bacterial strains of highest public health relevance. In some instances, MDR *E. coli* sequence types (e.g. ST10) frequently associated with infections were observed in irrigation water and on irrigated vegetables (Araújo et al., 2017).

Resistance to highly important antibiotics due to mobile ARGs was identified, although data on the pathogenic potential was generally not provided. The presence of MDR *E. coli* with resistance to extended spectrum cephalosporins has been described in manure from various animal species (poultry, pigs and dairy) and irrigation water (Guenther et al., 2017; Gekenidis et al., 2018). Moreover, resistance to colistin has also been reported in bacteria from pig manure (Guenther et al., 2017). Carbapenem resistant *E. coli* was reported in reused water, from effluent of sewage treatment plants, together with carbapenem- resistant *K. pneumoniae* and *Citrobacter freundii* (Zurfluh et al., 2017).

MDR and vancomycin resistant *E. faecium* and ciprofloxacin resistant *E. faecalis* were also reported in pig manure (Novais et al., 2013; Liu et al., 2018). Notably, similar strains of *Enterococcus faecium* resistant to vancomycin were detected in pig manure and in clinical samples (Freitas et al., 2011a). Carbapenem-resistant *A. baumannii* was also detected in pig manure (Hrenović et al., 2019).

Noteworthy is the presence of varied ARGs in manures and in contaminated irrigation water conferring resistance to CIAs, and that are common in clinical human isolates associated with mobile genetic elements, e.g. $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-27}}$, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-48}}$, $bla_{\text{NDM-5}}$ and $bla_{\text{VIM-1}}$ (Hrenović et al., 2019).

F.1.2. Plants-based food post-harvest: ARB and ARGs

Few surveys evaluating the occurrence and environmental sources of ARB and ARGs in warehouses and processing plants at the EU level are available. The May 2011 German outbreak of *Escherichia coli* O104:H4 carrying $bla_{\text{CTX-M-15}}$ (Bielaszewska et al., 2011), associated with fenugreek sprouted seeds, confirmed by trace back investigations (EFSA, 2011c) highlights the importance of good seed quality and the need for careful control and monitoring of the sprouting process.

F.2. Terrestrial animal production sector

The information collected on public health relevant bacteria and genes was summarised and included in Tables 8–10 (Sections 3.2.3.2–3.2.3.4).

F.2.1. Poultry production sector

Summarised information on public health-relevant ARBs and ARGs identified in the poultry production sector is presented in Table 8 (Section 3.2.3.2). A more detailed description of some relevant findings is presented below.

F.2.1.1. Poultry pre-harvest: ARB and ARGs

As previously indicated, poultry are considered to be an important source of common food-borne zoonoses. Pathogens that disseminate in the production environment, or which are intermittently introduced from the wider environment, are then widely disseminated via breeding pyramids and exchange between feed-mills, hatcheries, breeding, rearing and production farms, processing plants and egg packing centres (Hafez and Hauck et al., 2015; Van Meirhaeghe et al., 2019). Many of the zoonotic and commensal bacteria associated with poultry carry resistance to CIAs or have transmissible multiple drug resistance. The frequency of occurrence and distribution of such organisms within poultry production in Europe usually reflects the rigour of national control programmes for zoonoses, and AMR and is described in detail elsewhere (ECDC, EFSA and EMA, 2017b; EFSA and ECDC, 2021a,b).

All of the bacteria that can be found in the intestine of poultry, including antimicrobial resistant ones (Laube et al., 2014), will also be found in the poultry farm, transport and processing environments. This results in persistent contamination between flocks (Castañeda-Gulla et al., 2020), and contamination of faecal wastes, run-off or wash water, dust, food products and animal by-products such as raw meat pet food originating from poultry production. Furthermore, ARBs can be transferred to wild and feral animals and personnel associated with the farm (Vaz-Moreira et al., 2019). This can result in an ongoing cycle of transmission of ARB/ARGs between the contaminated environment and the animals within it.

One large European study on faecal samples taken at slaughter reported *mcr-1* in a number of poultry flocks, especially in Spain and Italy, which were identified as high colistin users at the time, as well as in Bulgaria. A high level of $bla_{\text{CTX-M-1}}$ was identified in Spain, Italy, Poland and Belgium. Plasmid-mediated quinolone (*qnr*) resistance was common in Polish and Bulgarian poultry (Munk et al., 2018).

Resistance to newer antimicrobials such glycylcyclines, ceftazidime/avibactam or oxazolidinones may become greater threats in future if transferrable resistance linked to fitness traits emerges in bacteria associated with poultry, or come from human sources and are co-selected through the common use of certain antimicrobials, such as tetracyclines or florfenicol (Freitas et al., 2011b; Wang et al., 2020a).

E. coli and other Enterobacterales such as *Klebsiella* spp. found in the farm environment (e.g. rats, flies, wild animals, manure/litter) are also often multidrug resistant, including to extended spectrum cephalosporins and fluoroquinolones, and some can persist in poultry farm and hatchery environments

(Solà-Ginés et al., 2015; Himsworth et al., 2016; Kaspersen et al., 2020a,b; Zhai et al., 2020) and spread between different flocks despite biosecurity precautions (Burow et al., 2020). Also of note is the presence of *E. coli* resistant to tetracycline, sulfamethoxazole, ampicillin and more rarely to aminoglycosides, chloramphenicol and fluoroquinolones in the few studies addressing private water supplies (Gilpin et al., 2020). Although the *E. coli* lineages or pathogenic potential were not evaluated, the transmissibility of genes encoding resistance to highly important antimicrobials confers a high priority to those microorganisms.

Campylobacter (*C. jejuni* and, to a lesser extent, *C. coli*) is the most common zoonotic organism associated with poultry and has the greatest economic impact (Dramé et al., 2020). *Campylobacter* spp. from caecal samples of broilers and fattening turkeys present an overall resistance to ciprofloxacin which is very high (over 70%). However, combined resistance – simultaneous resistance to two critically important antimicrobials – to fluoroquinolones and macrolides in *Campylobacter* remains low (EFSA and ECDC, 2021a,b). AMR profiles of *Campylobacter* are not available for most environmental isolates but are expected to include a high proportion of ciprofloxacin resistant *Campylobacter* as well as the usual intrinsic resistances (Castañeda-Gulla et al., 2020; Frosth et al., 2020). Drinking water and broiler transport crates have also been identified as sources of *Campylobacter,* including MDR/fluoroquinolone resistant strains (Peyrat et al., 2008; Coleman et al., 2013). Similar *Campylobacter* strains have been found in broilers and other animals on the farm in field studies suggesting that they can also perpetuate resistant *Campylobacter* strains on poultry farms (Ellis-Iversen et al., 2012).

A large proportion of *Salmonella* isolates of the most important serovars may not possess AMR, but there are important clones, such as *S*. Enteritidis, the most common zoonotic serovar, that may show fluoroquinolone resistance in high usage countries. Other clones of *S*. Typhimurium, *S*. Infantis, *S*. Kentucky, *S*. Newport and *S*. Heidelberg, showing fluoroquinolone and/or 3rd-generation cephalosporin resistance (EFSA and ECDC, 2021a), have been expanding worldwide by means of international trade in cheap eggs and poultry meat as well as global travel and transfer of infection from human sources (Hawkey et al., 2019; Bogomazova et al., 2020). MDR *Salmonella* Infantis and extended spectrum cephalosporin- resistant *S*. Heidelberg were observed, in a broiler farm, mice and workers (Elhariri et al., 2020). It is more likely that poultry workers acquire the bacteria from the poultry environment rather than being a source. Nevertheless, workers often move between different farms and there is circumstantial evidence that workers returning from foreign travel may rarely be a source of MDR *Salmonella* (Guillon et al., 2013). Of note is the recently emerged MDR clones of *S.* Heidelberg, *Salmonella* associated with greater risk for severe disease (Clothier and Byrne, 2016; Antony et al., 2018). AMR acquisition by several *Salmonella* serovars might be partially responsible for the enhanced virulence of certain strains and/or adaptation to different environments (Singer and Hofacre, 2006; Nde and Logue , 2008).

There is more limited evidence of emergence and environmental persistence of MRSA or *Acinetobacter* spp. resistant to multiple antimicrobials, including to extended-spectrum beta-lactams, fluoroquinolones and colistin. These can be found in poultry and related farm environments (Bortolaia et al., 2016; Carvalheira et al., 2017; Kittler et al., 2019; Ghaffoori Kanaan et al., 2020). Both are known to be very robust organisms, capable of prolonged survival outside the host (Makison and Swan, 2006; Gayoso et al., 2014).

Vancomycin resistant enterococci (VRE) in poultry received a great deal of attention after resistant strains acting as donors of resistance determinants to pathogenic strains were shown to be co-selected by the use of now-banned avoparcin. After the ban on avoparcin use in the EU in 1997, VRE occurrence in poultry reduced markedly. Increased rates of tigecycline- and oxazolidinone-resistant *E. faecium* strains have been reported in EU countries in recent years (DANMAP, 2015; Cavaco et al., 2017; MARAN, 2017; de Jong et al., 2019).

The resistances and genes/MGEs, including plasmid mediated ESBL/AmpC genes, in relation to poultry, are listed in Table 8, and discussed in detail in multiple publications (Apostolakos and Piccirillo, 2018; Antonelli et al., 2019; de Jong et al., 2019; EFSA, 2019; Roth et al., 2019; WHO, 2019a,b; Bogomazova et al., 2020; Mo et al., 2020; Racewicz et al., 2020). Other transferrable resistances to high priority therapeutic agents, such as carbapenems and colistin are currently rare in EU poultry populations in most countries (EFSA and ECDC, 2021a,b) but must not be allowed to become more widely established (Savin et al., 2020). One additional concern is the spread of transferable oxazolidinone resistance genes as their acquisition usually also confers resistance to phenicols and tetracyclines, common veterinary medicines which might enhance the burden of these ARGs in enterococci and staphylococci (Deshpande et al., 2018).

F.2.1.2. Poultry post-harvest: ARB and ARGs

All of the wide range of zoonotic and commensal bacteria found in poultry at farm level, and their associated resistances, will also be present at slaughter, either within the intestine or other organs of the birds or contaminating the integument and the slaughter plant equipment, air, waste and the wider environment (Haas et al., 2005; Sevilla-Navarro et al., 2020; Wieczorek et al., 2020). The resistome of poultry at slaughter is considered to be more diverse than for pigs, but with fewer gene copies (Munk et al., 2018). The microbial population of the slaughter plant environment is dominated by enteric commensal organisms, but also includes MDR zoonotic pathogens such as *Salmonella* with ESBL or AmpC genes, *Campylobacter* with high level fluoroquinolone resistance due to chromosomal mutations, MRSA and *L. monocytogenes* with clindamycin and biocide resistance, as well as highly MDR commensal organisms such as *E. coli*, occasionally with *mcr*-related colistin resistance (Oliveira et al., 2018; Feye et al., 2020; Iannetti et al., 2020). Other potentially MDR environmental bacteria such as *Pseudomonas, Clostridium, Psychrobacter* and (to a lesser extent) *Acinetobacter* spp. are abundant in the slaughter environment (Tsola et al., 2008; Chen et al., 2020).

Shackling, killing, plucking and evisceration of poultry are activities that contaminate air (Gregova et al., 2012; Pérez-Arnedo et al., 2020). Air samples containing bacteria with multi-drug resistance, including to extended-spectrum cephalosporins and fluoroquinolones were reported in both conventional and organic broilers slaughterhouses (Gregova et al., 2012; Musa et al., 2020). Another study on *Campylobacter jejuni* contamination reported high rates of resistance to ciprofloxacin, nalidixic acid and tetracycline, as well as to two common detergents used in the abattoir (García-Sánchez et al., 2019).

F.2.2. Cattle production sector

Summarised information on public health-relevant ARBs and ARGs identified in the cattle production sector is presented in Table 9 (Section 3.2.3.3). A more detailed description of some relevant findings is presented below.

F.2.2.1. Cattle pre-harvest: ARB and ARGs

The information available is mainly from research studies focusing on specific pathogens and not referring to specific production systems and their characteristics. Therefore we cannot for example in most instances determine if there was outdoor access.

For animals with access to the exterior, the pasture soil is a source of ESBL producing *E. coli* and highly important ARGs. $\textit{bla}_{\text{CTX-M}}$ genes have been found to persist in soil in farms and cultivated soil and pastures, for at least a year after the soils had been amended with manure (Hartmann et al., 2012). *E. coli* strains also encoding $\textit{bla}_{\text{CTX-M}}$ type genes ($\textit{bla}_{\text{CTX-M-1}}$, $\textit{bla}_{\text{CTX-M-2}}$, $\textit{bla}_{\text{CTX-M-14}}$ and $\textit{bla}_{\text{CTX-M-15}}$) on conjugative plasmids have been linked with environmental mastitis in dairy herds. It is likely that both clonal expansion of certain strains and horizontal transfer of plasmids have occurred (Freitag et al., 2017). The proximity of other farm animals may also be a factor for transmission. E.g. in organic dairy farms in the Netherlands, that had a pig farm within a radius of 2 km of the cattle barn significantly increased the probability of ESBL/AmpC *E. coli* being found in the herd (Santman-Berends et al., 2017). The presence of waterfowl within 1 km of German broiler, pig, dairy or beef cattle farms was associated with changes in the ESBL gene families and the resistance phenotypes of cefotaxime-resistant *E. coli* in farm animals and in wild game in the area surrounding the farm (Hille et al., 2018a). Vectors such as arthropods may also contribute to dissemination of resistant *E. coli*; overlapping resistance genes and PFGE patterns were identified in calves and in flies from a dairy farm, suggesting a possible role of flies as vectors for dissemination of ARB (Rybaríková et al., 2010). The plasmid-mediated colistin-resistance gene *mcr-1* was found in *E. coli* that also carried an ESBL gene in floor faecal samples within French calf units (Haenni et al., 2016).

Air and dust have also been identified as sources for MRSA (Hordijk et al., 2019) and ESBL-producing *E. coli* (Schmid et al., 2013). The immediate farm environment, assessed by testing environmental samples (feed troughs, water troughs, milk buckets, automatic feeders, pen walls and pen floors), was found to contribute to colonisation of calves by quinolone-resistant *E. coli* in a Swedish study that concluded that farms show different levels of prevalence of quinolone resistance (Duse et al., 2016). Similarly, cefotaxime-resistant bacteria were found in forage used on outdoor beef cattle farms (Markland et al., 2019). Resistance has been observed in the absence of selective pressure perhaps because specific antimicrobial resistant strains and or genetic elements might be spreading to soils and crops and, from there, further disseminated by wildlife (O'Brien, 2002).

Transport and overcrowding of veal calves from different origins in fattening units is known to lead to increased introduction of diverse ARB/ARGs from different origins, including ESBLs and occasionally colistin resistance (Gay et al., 2019).

There are both general and host-associated lineages of antimicrobial resistant *S.* Typhimurium. E.g. in a UK study, the diversity of AMR profiles was observed to be greater in pig than in cattle or chicken isolates, and, among cattle, diversity was greater in beef cattle isolates than in those from dairy cattle. This suggests different sources and/or selection pressures between the species and sectors (Mellor et al., 2019). *S*. Typhimurium isolates from cattle in the same study showed a high prevalence of MDR (74.4%). Even though there were some overlaps of AMR profiles between isolates of cattle origin and other domestic species, 4.6% profiles were exclusive to cattle. Moreover, WGS information did not always directly link to resistance profiles and in several instances similar profiles were found across different lineages (Mellor et al., 2019).

There is a paucity of data on resistance of environmental isolates of *Campylobacter* species. In general *Campylobacter* is found in the gut flora of different food and wild animal species, including cattle, but also show some degree of host specificity. Environmental contamination is also associated with water sources (Ogden et al., 2009).

MRSA is a concern in the milk production chain. MRSA ST398, CC97-carrying *mecA* and the *mecC* containing lineages CC130 and ST425 are robust organisms that resists desiccation and harsh environments and are therefore able to persist in the farm environment for long periods (up to years) years and infect the mammary glands of dairy cows (Barberio et al., 2019). Use of antimicrobials such as tetracyclines and beta-lactams has been mentioned as a factor favouring their persistence. Furthermore, co-selection due to resistance to biocides such as *czrC* genes found frequently in the SCCmec close to *mecA* gene in CC398 isolates may be associated with persistence in farms (Cavaco et al., 2011). MRSA ST398 in cattle in the Netherlands has been associated with farms housing both cows and pigs and therefore it is likely that inter-species transmission has occurred (Tavakol et al., 2012). This has also likely happened in e.g. Denmark and Germany, as it appears that MRSA in cattle has largely originated from a spillover from pig production, as most isolates clustered within the main clonal lineages found in pigs (Hansen et al., 2019) and with the farm environment contributing as to exchange of ARBs (Fessler et al., 2012). Moreover, some lineages can also originate or persist in other reservoirs, such as hedgehogs (Rasmussen et al., 2019). For some other genotypes which cluster outside these lineages, other sources such as human introduction are likely (Hansen et al., 2019).

Metagenomic AMR studies concerning cattle in Europe have unfortunately not included environmental samples. Several studies have shown that cattle have a relatively dynamic faecal microbiota in the early stages of life which is influenced by colostrum intake and the environment and developing after weaning while gradually adapting to changing feeding practices and maturity. Younger animals have higher relative abundance of ARG elements in general which reduce with age, impacting the environment (Noyes et al., 2016; Liu et al., 2019).

F.2.2.2. Cattle post-harvest: ARB and ARGs

Few studies are available on the post-harvest environment and these do not focus directly on AMR in relation to cattle slaughter. However, cattle, and especially veal calves, are significant reservoirs for ESBL and MCR - producing *E. coli* isolates (Hernández et al., 2017). E.g. calf samples taken at slaughter in France reflects the dissemination of a variety of *bla*$_{\text{CTX-M}}$, likely originating from different animal and environmental contamination pathways (Haenni et al., 2014).

F.2.3. Pig production sector

Summarised information on public health-relevant ARBs and ARGs identified in the pig production sector is presented in Table 10 (Section 3.2.3.4). A more detailed description of some relevant findings is presented below.

F.2.3.1. Pig preharvest: ARB and ARGs

Pigs are reservoirs for several pathogens of human relevance including *Salmonella,* MRSA, *Campylobacter* and *Enterococcus*. While *Salmonella* can be pathogenic for pigs (Fedorka-Cray et al., 1994), pigs are merely carriers or vectors of other relevant pathogens such as MRSA, *Campylobacter* spp., *Listeria* spp. or *Enterococcus* spp.

Salmonella are noteworthy because of the high prevalence of this pathogen in the pig production chain (EFSA, 2008c, 2009a) and the control plans established in several European countries (Osterkorn et al., 2001; Quirke et al., 2001; Christensen et al., 2002). More than 50 serovars have been described in pigs (EFSA, 2008c, 2009a) but the most prevalent are *S*. Derby, *S*. Typhimurium and its monophasic variant *S.* 4[5],12:i:- (EFSA, 2008c, 2009a,b; Hopkins et al., 2010). Features enhancing colonisation, adhesion, intestinal invasion, survival in host tissues, biofilm formation, the ability to survive acidic intestinal pH and organic acid supplementation of feed, and heavy metal tolerance, are attributes associated with these pig- associated serovars (Campos et al., 2019). *Salmonella* AMR varies by serovar and between clonal groups (Beutlich et al., 2011), although the presence of AMR and multi-resistance is quite frequent in *S*. Typhimurium and *S*. 4[5],12:i:-. *S*. 4,12,:i:- strains with AMR to critically important antibiotics such as ciprofloxacin have been found in isolates from the pig feed mill production environment and/or raw feed ingredients (Burns et al., 2015). Mammalian and avian wildlife also represent a threat for the introduction of AMR *Salmonella* into the food chain (de Lucia et al., 2018). E.g. various serovars carrying AMR and mobile genetic elements have been isolated from wild boar (Caleja et al., 2011; Zottola et al., 2013; Leekitcharoenphon et al., 2019). The outdoor environment may contain MDR *Salmonella* with resistance to critically important antibiotics such as cefotaxime and ceftazidime (Fischer et al., 2017). Survival of MDR *S*. Typhimurium after inadequate cleaning and disinfection of pig housing and equipment, resulting in increased infection of pigs, can occur (Martelli et al., 2017).

The presence of MRSA on pig farms has also been extensively studied (EFSA, 2009b), revealing the frequent isolation of this pathogen and particularly of the ST398 lineage able to colonise humans and cause infection (EFSA, 2009b; Cuny et al., 2015; Peeters et al., 2015; Lopes et al., 2019). Transmission of MRSA (e.g. CC398) to and from humans has also been reported in a significant proportion of humans with occupational exposure to pigs (Cuny et al., 2015). Apart from pigs' skin and faeces, which are vehicles for the pathogen, it can be detected in dust or bioaerosols (Pilote et al., 2019). Environmental MRSA carrying the *mecA* gene and exhibiting resistance to ciprofloxacin, has been described (Argudín et al., 2011; Ruiz-Ripa et al., 2020). Moreover, different MRSA lineages, including ST398, have been reported in different wild animals across Europe (Heaton et al., 2020).

E. coli isolates carrying *mcr* genes are often resistant to other CIAs such as carbapenems (Fischer et al., 2013, 2017; Borowiak et al., 2017; Pulss et al., 2017; Roschanski et al., 2017b; Hille et al., 2018b; Rebelo et al., 2018). Of relevance is the detection of AMR resistance to 3rd-generation cephalosporins (Cameron-Veas et al., 2015; Lalak et al., 2016; Aguirre et al., 2020), which, as a result of the reduction or discontinuation of the use of these antimicrobials in pig production, seem to be following a decreasing trend (Agersø and Aarestrup, 2013; Bourély et al., 2018). Although restricted to a limited number of farms, carbapenem-resistant *E. coli* have been detected in manure, flies, or inside animal housing on commercial pig farms in Germany (Fischer et al., 2017), which demonstrates the spread of ARGs after their detection in faeces (Fischer et al., 2013). Previously, Usui et al. (2015) reported a link between antimicrobial resistant *E. coli* from flies and pig faeces, although for isolates that were not resistant to critically important antimicrobials.

The main *Campylobacter* species isolated in pigs is *C. coli*, which is estimated to be involved in 5% of human campylobacteriosis cases (Kempf et al., 2017; Rossler et al., 2019). *Campylobacter coli* strains resistant to ciprofloxacin have been described in conventional and antibiotic-free production pig herds (Quintana-Hayashi and Thakur, 2012). Despite the vast literature on *Campylobacter* and livestock, including AMR, in particular to fluoroquinolone resistance (Wang et al. 2016), there are very few publications evaluating the presence of *Campylobacter* spp. on pig farms environments (Alter et al., 2005; Jensen et al., 2006) and no reference to AMR. Environmental *Campylobacter* presumably carry the same ARGs observed in faecal isolates, but this needs to be confirmed.

Enterococcus spp., are used as indicator bacteria in AMR monitoring in pigs (Rizzotti et al., 2005; Braga et al., 2013; Novais et al., 2013) 2020/1729[74]). *Enterococcus faecium* and *E. faecalis* are the two most frequent species isolated on pig farms (Novais et al., 2013). Detection of VRE is particularly important (Braga et al., 2013; Novais et al., 2013) but a high prevalence of *Enterococcus* spp., resistant to gentamicin or ciprofloxacin has also been reported within the pig farm environment (Novais et al., 2013).

Some of the studies already mentioned in the sources and pathways section or in the previous paragraph have further characterised the genes involved in AMR resistance. Thus, the *bla*$_{\text{VIM-1}}$

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[74] Commission Implementing Decision (EU) 2020/1729 of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria and repealing Implementing Decision 2013/652/EU (notified under document C (2020) 7894). OJ L 387, 19.11.2020, p. 8–21.

carbapenem resistance gene has been described in *Salmonella* isolated from the farm external environment (Fischer et al., 2013, 2017). Within the EU, specific carbapenemase-monitoring (EFSA and ECDC, 2021a) was performed in 2019 and *E. coli* producing *bla*$_{\text{CTX-M}}$ genes have been described across EU (Cameron-Veas et al., 2015; Lalak et al., 2016; Aguirre et al., 2020), while other genes such as *bla*$_{\text{OXA-48}}$ and *bla*$_{\text{GES}}$ carbapenemases appear to be more restricted to particular farms. These two genes were found in German pig herds and both cases were further investigated; environmental samples were analysed, but no further positive isolate was found (Irrgang et al., 2020). Genes encoding resistance to CIAs (e.g. colistin) have been observed in *K. pneumoniae* from faecal waste/manure from pigs (Kieffer et al., 2017; Fournier et al., 2020). Nevertheless, the presence of CIAs-resistant *K. pneumoniae* or *Acinetobacter* species may be underestimated due to the difficulty in culturing these microorganisms in commonly used culture media.

Other genes detected in pig farm environmental samples include the β-lactamases *bla*$_{\text{PSE-1}}$ (gene associated with the *Salmonella* genomic island 1, SGI-1) and *bla*$_{\text{TEM}}$ present in *S.* 4[5],12:i:- (Tassinari et al., 2019). Genes such as *erm*(B) which confer resistance to MLS phenotype or *aac(6′)* aminoglycoside resistance genes have been detected in *Enterococcus* spp., isolated from pig farm dust, housing or drinking water (Novais et al., 2013). Also, in dust samples or bioaerosols, genes such as *bla*$_{\text{CTX-M-1}}$, or *mcr-1* have been detected by PCR (Pilote et al., 2019). Studies in wild boar have reported the presence of MDR *Salmonella* serovars carrying genes such as *bla*$_{\text{TEM-1}}$ (Leekitcharoenphon et al., 2019) or *bla*$_{\text{PSE-1}}$ gene cassettes (Caleja et al., 2011) within class 1 integrons.

Recent international studies have characterised the pig faecal resistome (Xiao et al., 2016; Munk et al., 2018; Wang et al., 2019) or the EU farm environment (Luiken et al., 2020; Mencía-Ares et al., 2020). ARGs in both dust and the farm environment are more diverse compared to the pig faecal resistome. Perhaps this is due to the microbial shift occurring in an aerobic environment, which facilitates the growth of faecal Enterobacterales and soil-based organisms including *Streptococcaceae*, *Bacteriodaceae*, *Peptostreptococcaceae*, *Staphylococcaceae* and *Clostridiaceae,* which accounted for 58.5% of the total ARG abundance and 75.8% of all assigned ARGs (Mencía-Ares et al., 2020). Analysis of mobile genetic elements within this study also revealed a higher abundance of MGEs and plasmids in environmental samples from pig farms as compared to other farms (Mencía-Ares et al., 2020).

F.2.3.2. Pigs post-harvest: ARB and ARGs

There are some international studies, including European ones, which have evaluated the presence of food-borne pathogens such as *Salmonella* spp., MRSA*, L. monocytogenes* or *E. coli* in pig slaughterhouse environments, as mentioned in Section 3.1.2. The presence of *Salmonella spp.,* in the pig food chain is well documented by a number of studies, some of which have focused on post-harvest stages. Thus, Walia et al. (2017) reported the AMR profiles of serovars isolated from lairage environment in a cleaning and disinfection study. Among the AMR phenotypes, there were strains with MDR to tetracyclines, 2nd-generation cephalosporins (cefoxitin), aminoglycosides such as gentamicin and other antimicrobials such as chloramphenicol. Despite the large number of studies evaluating the presence of *Salmonella* on carcasses and abattoir environment (van Hoek et al., 2012; Argüello et al., 2013a), only a few have analysed the AMR of environmental strains, and AMR phenotypes to last resort antimicrobials have not been recorded in *Salmonella* isolated from the abattoir environment in the EU, despite human occupational acquisition of ESBL producing *E. coli* (Dohmen et al., 2017). The frequent isolation of resistant and multi-resistant isolates from carcasses (Bolton et al., 2013; García-Fierro et al., 2016; Fois et al., 2017) would suggest that similar resistant profiles can be found in the slaughterhouse environment (machinery, workers hands and implements, etc.), and there is a connection between *Salmonella* present on carcasses and slaughter activities (Giovannacci et al., 2001; Argüello et al., 2013a; Gomes-Neves et al., 2014). Two studies describe the isolation of MRSA resistant to gentamicin in the slaughterhouse environment (Van Cleef et al., 2010) and particularly in workers involved in scalding and de-hairing activities (Gilbert et al., 2012).

In addition to culture-based studies, there are some studies evaluating occupational exposure to ARGs such as *tet*W or *ermB* which confer resistance to tetracycline and macrolides respectively (Van Gompel et al., 2020). Both genes and antimicrobial classes are among the most abundant in pig faecal resistomes (Munk et al., 2018). The study of Van Gompel and colleagues demonstrated the dissemination of these two genes, which were found in samples collected from workers, air and abattoir environments, with exposure to both genes in the slaughter line.

F.3. Aquaculture production sector

Summarised information on antimicrobial-resistant bacterial species identified in aquaculture, both human and fish pathogens, is presented in Table 11 (Section 3.2.3.5) and Tables F.1–F.2. (Appendix F). A more detailed description of some relevant findings is presented below.

F.3.1. Aquaculture preharvest: ARB and ARGs

There is a small amount of literature on AMR in highest priority bacteria, including reports of tetracycline, macrolide and beta-lactam resistance in *Enterococcus* spp. including *E. faecium* isolated from sediments and seawater from Mediterranean aquaculture sites (Di Cesare et al., 2012). *Enterococcus* spp. were also isolated from rainbow trout in Spain and Portugal, which demonstrated resistance to at least one antimicrobial (Araújo et al., 2015; Novais et al., 2018). *K. pneumoniae* harbouring fluoroquinolone resistance genes (*qnrB7, oqxA* and *oqxB*) was reported from sediment and feed on a Portuguese trout farm (Antunes et al., 2018). Concerning *Salmonella*, a study of trout farms in Portugal reported several *Salmonella* serovars, including g *S*. Newport-ST118, *S*. Linguere-ST508, *S*. Guerin-ST508 and *S*. Abony-ST1672. All were detected in diverse samples of water, sediment and/or fish. Resistance was observed to streptomycin and kanamycin in some isolates and colistin resistance was reported from *S.* Abony due to mutations in *pmrA* and *pmrB* (Antunes et al., 2018*)*. A study of Spanish shellfish in 2004 identified *S*. Typhimurium resistant to multiple antimicrobials, including two DT4 isolates resistant to 8 and 7 antimicrobials (Martínez-Urtaza et al., 2004). A more recent large study of mussels sampled from 15 production areas and four processing facilities in Spain resulted in the isolation of 19 *Salmonella* strains including *S.* Montevideo-ST316, *S.* Rissen-ST469, *S.* Wentworth-ST2031, *S.* Typhimurium-ST34, *S.* Typhimurium-ST19, *S.* Typhimurium-STnew, *S. S.* Liverpool-STnew, *S.* Liverpool-ST-1959, *S.* Senftenberg-ST14 and *S.* Bredeney-ST306 from raw mussels. Phenotypic AMR was reported to between 3 and 7 antimicrobials, including cephalosporins, gentamicin, amikacin, trimethoprim/sulfamethoxazole, nalidixic acid and ciprofloxacin, amongst others (Lozano-León et al., 2019). Of note, genome sequencing of *Salmonella* Rissen ST469 revealed *aac(6′)-Iaa*, *aadA1*, *aadA2*, *bla*$_{\text{TEM-1B}}$, *cmlA1*, *sul1*, *sul3*, *tet*(A), *dfrA1* and *mcr-1* (Lozano-León et al., 2019).

There are a large number of antimicrobial resistance determinants found in fish pathogens and other marine and freshwater bacteria (Tables F.1 and F.2) that are shared with human pathogens, including plasmid-mediated fluoroquinolone resistance genes, macrolide and tetracycline resistance genes (Cabello et al., 2016). Moreover, these bacteria can act as reservoirs for possibly emerging genes (e.g. *mcr* genes) (Yin et al., 2017; Ling et al., 2020).

Due to the small amount of data available for priority pathogens, a separate systematic approach was applied to reviewing the presence of ESBL and carbapenamase genes in ‘other’ bacterial species from aquatic environments and aquaculture production systems. Some attention was given to genes reported in aquatic environments and in wild fish and shellfish, given the extremely small number of studies focusing on farmed fish and shellfish (Roschanski et al., 2017a).

The literature searches performed (Appendix C) confirmed that a limited amount of research has been undertaken but that data available suggests that gram-negative pathogens carrying ESBLs are present in both finfish and shellfish produced in Europe. Due to the small amount of available literature and the difficulty of attributing environmental origins of ARB from point of sale fish and shellfish produced in Europe, studies of wild fish and shellfish have been included as they may represent useful proxies for farmed fish in the absence of data on AMR in aquaculture production systems.

There is a limited body of literature on the presence of extended spectrum beta-lactamase (ESBL) producing bacteria in fish, shellfish and aquatic environments; however, it is known that wastewater pollution introduces ESBL producing opportunistic gram-negative pathogens into European freshwater and marine systems (Amos et al., 2014; Leonard et al., 2015). It has also been shown that direct human exposure to aquatic environments is associated with exposure to and colonisation by ESBL - *E. coli* (Leonard et al., 2018) and direct exposure to fresh waters are associated with increased probability of suffering from UTI infections caused by ESBL - *E. coli* (Søraas et al., 2013a,b). In a Norwegian study (Søraas et al., 2013a, 2013b), eating fish had a protective association with UTI infections caused by ESBL-*E. coli,* whereas Mughini-Gras et al. (2019) found a positive association between seafood consumption and colonisation by ESBL *E. coli* in the Netherlands. Among other possible factors, the authors pointed out that the high consumption of imported seafood in the Netherlands (also from areas where antimicrobials are commonly use) could account for the association reported.

A study of wild fish from the Algerian Mediterranean Sea reported carriage of ESBL- producing *E. coli*, *K. pneumoniae*, *Enterobacter cloacae* complex, *Morganella morganii*, *Citrobacter freundii* complex and *Proteus vulgaris* in 21.3% (64/300) fish samples predominantly bearing $bla_{\text{CTX-M.}}$ The area sampled is presumably contaminated with untreated human sewage (Brahmi et al., 2018). A study of wild freshwater fish in France reported ESBL - *E. coli* in 0–85% of fish depending on sample site and species and this was associated with proximity to human faecal pollution (Bollache et al., 2019). Studies have also focused on wild gilthead sea bream in Portugal, where 5/118 (4.2%) faecal samples contained ESBL-positive *E. coli* which carried $bla_{\text{TEM-52}}$ or bla_{SHV-12} genes (Sousa et al., 2011).

Shellfish can be contaminated by ARB present in human and livestock waste entering aquatic systems; however, this is a function of aquatic pollution and is largely outside the control of shellfish producers. Wild-grown Mediterranean mussels produced in human-impacted waters in Croatia were reported to contain *Pseudomonas aeruginosa* resistant to 3rd-generation cephalosporins carrying $bla_{\text{TEM-116}}$ (Maravić et al., 2018). Further studies of wild-growing Mediterranean mussels demonstrated carriage of *Aeromonas* spp. isolates with $bla_{\text{CTX-M-15}}$ and/or $bla_{\text{SHV-12}}$ or $bla_{\text{FOX-2}}$ gene (Maravić et al., 2013). Shellfish are often subject to depuration procedures to reduce microbial contamination; however, ESBL-producing *Enterobacteriaceae* were present in retail shellfish, including those likely to be from aquaculture production systems, originating in Denmark, France, Ireland and Italy, with mussels, clams and cockles testing positive for ESBL producing Enterobacteriaceae (Vu et al., 2018). In a large study, bivalve samples were collected from localities covering the Norwegian coast, mostly from commercial rearing sites, with the remainder being from long term reference monitoring locations (Grevskott et al., 2017). At least 75% of the Enterobacterales isolates tested showed resistance to one or more antimicrobial; two *E. coli* isolates showed resistance to 3rd-generation cephalosporins associated with the co-presence of $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$.

This review of the literature highlights the extremely small number of studies focusing on ESBL-producing bacteria in aquaculture production systems; however, it does show that both European finfish and shellfish production systems have reported *ESBL*-producing bacteria and that in a small number of cases this could be identified as being associated with human faecal pollution. More attention has been given to bivalve shellfish due to their capacity to filter large volumes of water and accumulate human pathogens. The fact that bivalves are often eaten raw or lightly cooked also increases the likelihood of transmission of ARB to humans.

Clinically important carbapenem resistance genes (e.g. $bla_{\text{VIM-1}}$, bla_{NDM}, bla_{KPC}, $bla_{\text{OXA-48}}$) are widely reported in bacteria from freshwater or seawater sources in Europe (Mahon et al., 2017; Bleichenbacher et al., 2020; Hooban et al., 2020). A recent systematic review by Bonardi and Pitino (2019) focused on carbapenemase-producing bacteria in food-producing animals, including fish and molluscs, but only one report in wild venus clams collected from retail in Germany, and originating from the Italian Mediterranean was included, with $bla_{\text{VIM-1}}$ described in *E. coli* (Roschanski et al., 2017a). However, other studies have characterised carbapenemases in aquaculture environments, including a study of *Aliivibrio salmonicida* (previously known as *Vibrio salmonicida*), a well know fish pathogen which has a chromosomally encoded metallo beta-lactamase (MBL), $bla_{\text{ALI-1}}$ conferring carbapenem resistance belonging to the betalactamases subclass B1, and sharing 39% and 29% amino acid identity with $bla_{\text{IMP-1}}$ and $bla_{\text{VIM-2}}$, respectively. Other papers on carbapenemases in *Aeromonas* spp. indicate that *Aeromonas hydrophila* and *Aeromonas veronii* (human opportunistic pathogens ubiquitous in the aquatic environment) associated with aquaculture (Smyrli et al., 2019), also carry a class B MBL *cphA* which is not easily detected by conventional *in vitro* susceptibility tests (Chen et al., 2012). However, in the presence of a carbapenem antibiotic, increased production of the CphA enzyme can lead to resistance. *A. hydrophila* is considered an emerging human pathogen responsible not only for gastroenteritis and skin infections, but also for more serious systemic conditions such as peritonitis, bacteraemia, meningitis, cholera-like illness, haemolytic uraemic syndrome and necrotising fasciitis (Citterio and Biavasco, 2015).

In conclusion, whilst there are few reports of well-known clinical carbapenemases in bacteria from European aquaculture production systems, this is likely to be due to their relative rarity combined with lack of coordinated surveillance, and a focus on Enterobacterales. It is clear from the literature that opportunist pathogens of both fish and humans belonging to indigenous aquatic groups including *Aliivibrio salmonicida* and *Aeromonas spp*. can be reservoirs of carbapenemases and are widespread in aquaculture production environments.

F.3.2. Aquaculture post-harvest: ARB and ARGs

As mentioned in Section 3.1, only a few studies have looked at the presence of ARB or resistance determinants during post-harvest processing of fish and shellfish, or attempted to identify the sources and pathways of such contamination, and most of these were from countries outside Europe. These studies included detection of antimicrobial resistant *L. monocytogenes* (Noll et al., 2018; Skowron et al., 2018).

Table F.1: Best known antimicrobial-resistant bacteria in aquaculture

Bacteria(a)	**Occurrence on fish (meat)**	**Occurrence in shellfish – shrimp (intestines)**	**Effect on environment**	**Effect on human exposure**	**Supporting references**
Salmonella enterica	+	+	Not naturally in fish microbial flora, introduced through contaminated water/poor hygiene. Survives in soil and water. Spread in environment.	*S. enterica*: human pathogen causing gastroenteritis, bacteraemia, etc.	Fernandes et al. (2018), Antunes et al. (2018), Lozano-León et al. (2019)
***Enterococcus* spp.** *(E. faecium, E. faecalis, E. hirae, E. casseliflavus, E. gallinarum)*	+		Not naturally in fish microbial flora, introduced through contaminated water/poor hygiene, reservoir in coastal marine environments and other aquatic systems, enrichment in sediments.	Human opportunistic pathogen; some lineages seen in nosocomial infections.	Barros et al. (2011), Di Cesare et al. (2013), Novais et al. (2018)
E. coli	+	+		Important human commensal. Opportunistic or pathogenic potential for some phylogenetic lineages.	Bos et al. (2016), Brisabois et al. (2019)
***Pseudomonas* spp.** *(P. fluorescens, P. aeuruginosa)*	(+)	+	Enrichment in sediments, presence in mussels.	Some species are human opportunistic pathogen.	Haenen (2017), Piotrowska et al. (2017), Scales et al. (2014), Brisabois et al. (2019)
***Aeromonas* spp.** *(A. salmonicida,* A. sobria, *A. hydrophila, A. caviae, A. veronii)*	+	+	Naturally occurring in water environments, can serve as reservoirs of ARGs, enrichment in sediments.	Can probably transfer ARGs to human pathogens such as *E. coli*. *A. hydrophila* complex, *A. caviae, A. veronii:* gastrointestinal and extraintestinal infections.	Alcaide et al. (2005), Cabello (2006), Čížek et al. (2010), Naviner et al. (2011), Schmidt et al. (2000), Smyrli et al. (2017), Stratev et al. (2015), Xia et al. (2010), Brisabois et al. (2019), Zdanowicz et al. (2020)
***Vibrio* spp.** *(V. alginolyticus, V. anguillarum, V. harveyi, V. splendidus, V. Scophthalmi, V. mimicus, V. furnissii)*	+	+	Naturally occurring in marine and estuarine environments, can serve as reservoirs of ARGs.	*V. alginolyticus:* wound infections, otitis media, otitis externa and gastrointestinal infections. *V. cholerae, V. vulnificus* and *V. parahaemolyticus:* human pathogen.	Ripabelli et al. (2003), Alcaide et al. (2005), Rodríguez-Blanco et al. (2012), Elbashir et al. (2018), Brisabois et al. (2019)
Flavobacterium psychrophilum	(+)		Enrichment in sediments.	Fish pathogen.	Schmidt et al. (2000), Haenen (2017)

Bacteria[(a)]	**Occurrence on fish (meat)**	**Occurrence in shellfish – shrimp (intestines)**	**Effect on environment**	**Effect on human exposure**	**Supporting references**
Yersinia ruckeri	+		Survive without host in water, sediments and biofilms, can spread in environment.	Fish pathogen.	Huang et al. (2015), Haenen (2017)
Edwardsiella spp**.** *(E. tarda/E. piscicida,* *E. ictaluri/E. anguillarum)*	+		Naturally in aquatic environments (survive without host in pond water and mud) remains in carrier position in other organisms (mammals, birds, amphibians, aquatic invertebrates).	*E. tarda, E. piscicida:* human pathogen (causing bacteraemia).	Mohanty and Sahoo (2007), Katharios et al. (2015), Haenen (2017), Hu et al. (2018)
Stenotrophomonas spp.		+	Naturally occurring in aquatic/ humid environments, can serve as reservoirs of ARGs.	*S. maltophilia:* human opportunistic pathogen.	Looney et al. (2009), Piotrowska et al. (2017), Brisabois et al. (2019)

(a): In bold, antimicrobial-resistant bacteria of highest priority for public health as described in 3.2.2.

Table F.2: Examples of resistance genes in bacteria detected in fish and shellfish

Resistant bacteria (ARBs)$^{(a)}$	Species	Resistance genes (ARGs)	Reference
Salmonella spp. *S. enterica*	European eel Mussel	*mcr-1, sul1, sul2, sul3, fosA, fosA7, aadA1, aadA2,* blaTEM-1B, *mph(A), tet*(A), *tet*(B), *dfrA1, dfrA12, cmlA1, aph(6)-Id, aph(3″)-Ib, aac(6′)-Iaa*	Alcaide et al. (2005), Lozano-León et al. (2019)
Enterococcus spp. *(E. faecium, E. faecalis, E. hirae, E. casseliflavus, E. gallinarum)*	Rainbow trout, sea bass, sea bream	*tet*(M), *tet*(L), *tet*(S), *erm(B), cat(pC223), cat (pC221), cat(pC194), fexB, aadE, aac(6′)-le-aph(2″)-Ia* (from water, sediment, trout and feed)	Di Cesare et al. (2013)
E. coli	Mussel	*bla*$_{\text{TEM-1B,}}$ *bla*$_{\text{CYX-M-15,}}$ *bla*$_{\text{CTX-M-14,}}$ *strA-strB, dfrA5, dfrA14, dfrA17, sul1, sul2, qnrS1, tet(A), tet(B), tet* (D), *catA1, aac(3)-lld, aph (3′)-la, mph(A), aadA5.*	Grevskott et al. (2017)
Pseudomonas spp	Carp	*tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(M), *tet*(S), *tet*(T), *tet*(30), *tet*(32), *tet*(34), *str(A), str(B), aac(6′)-I, erm (C), erm(E), erm(F), erm(V), erm(X)* (from water)	Piotrowska et al. (2017), Preena et al. (2020)
Aeromonas spp. *(A. Salmonicida, A. sobria, A. hydophila, A.veronii)*	Atlantic salmon, rainbow trout, European whitefish, carp, sea bass, European eel	*tet*(E), *tet*(A), *tet*(D) (from gill and skin)	Schmidt et al. (2000), Alcaide et al. (2005), Čížek et al. (2010), Naviner et al. (2011), Smyrli et al. (2017), Miller and Harbottle (2018), Preena et al. (2020)
Vibrio spp. *(V. vulnificus, V. alginolyticus, V. harveyi, V. anguillarum, V. splendidus, V. scophthalmi, V. mimicus, V. furnissii)*	Mediterranean mussel, Gilthead sea bream, European eel, European sea bass, Turbot, Sole	*tetA*	Ripabelli et al. (2003), Alcaide et al. (2005), Rodríguez-Blanco et al. (2012), Scarano et al. (2014), Brisabois et al. (2019), Miller and Harbottle (2018)
Flavobacterium spp. (Flavobacterium psychrophilum)	Atlantic salmon, rainbow trout, carp	(resistance to quinolones but no *qnr* genes/no plasmid-mediated quinolone resistance). *tet*(B), *tet* (O), *tet*(T), *tet*(I), *tet*(32), *str*(A), *aac*(6′)-I, *erm*(F), *msr*(A)	Schmidt et al. (2000), Shah et al. (2012a,b), Piotrowska et al. (2017)
Yersinia ruckeri	Atlantic salmon, rainbow trout	(resistance to quinolones but no qnr genes/no plasmid-mediated quinolone resistance)	Schmidt et al. (2000), Shah et al. (2012a,b), Miller and Harbottle (2018)
Edwardsiella spp.	European eel, European sea bass, turbot, sole, European whitefish, Sharpsnout sea bream	Phenotypic resistance to ampicillin and sulfamethoxazole/trimethopirm	Katharios et al. (2015), Miller and Harbottle (2018), Buján et al. (2018)
Stenotrophomonas	Carp	*tet*(B), *tet*(C), *tet*(D), *tet*(M), *tet*(X), *tet*(32), *tet*(34), *strA*, *strB*, *aac(6′)-I*, *msr*(A), *erm*(C), *erm*(X) (from water)	Piotrowska et al. (2017)

(a): In bold, antimicrobial-resistant bacteria of highest priority for public health as described in 3.2.2.