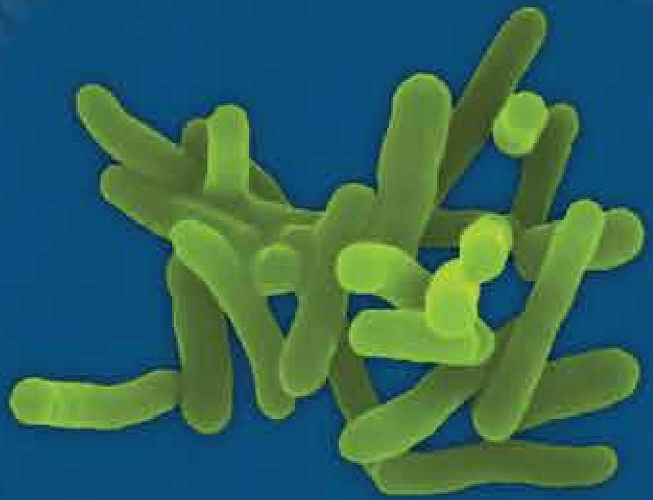
ISSN 1726-5274

Food and Agriculture
Organization of the
United Nations

World Health
Organization

Joint FAO/WHO Expert Meeting in collaboration with OIE on Foodborne Antimicrobial Resistance: Role of the Environment, Crops and Biocides

MEETING REPORT



34

MICROBIOLOGICAL RISK
ASSESSMENT SERIES

34

MICROBIOLOGICAL RISK
ASSESSMENT SERIES

Joint FAO/WHO Expert Meeting in collaboration with OIE on Foodborne Antimicrobial Resistance: Role of the Environment, Crops and Biocides

MEETING REPORT

Food and Agriculture Organization of the United Nations
World Health Organization

Rome, 2019

Required citation:
FAO and WHO. 2019. Joint FAO/WHO Expert Meeting in collaboration with OIE on Foodborne Antimicrobial Resistance: Role of the Environment, Crops and Biocides – Meeting report. Microbiological Risk Assessment Series no. 34. Rome.

The designations employed and the presentation of material in this information product do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations (FAO) or the World Health Organization (WHO) concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or products of manufacturers, whether or not these have been patented, does not imply that these have been endorsed or recommended by FAO or WHO in preference to others of a similar nature that are not mentioned.

The views expressed in this information product are those of the author(s) and do not necessarily reflect the views or policies of FAO or WHO.

ISBN 978-92-5-131890-4 (FAO)
ISBN 978-92-4-151690-7 (WHO)
© FAO and WHO, 2019

Some rights reserved. This work is made available under the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 IGO licence (CC BY-NC-SA 3.0 IGO; https://creativecommons.org/licenses/by-nc-sa/3.0/igo/).

Under the terms of this licence, this work may be copied, redistributed and adapted for non-commercial purposes, provided that the work is appropriately cited. In any use of this work, there should be no suggestion that FAO or WHO endorses any specific organization, products or services. The use of the FAO or WHO logo is not permitted. If the work is adapted, then it must be licensed under the same or equivalent Creative Commons license. If a translation of this work is created, it must include the following disclaimer along with the required citation: “This translation was not created by the Food and Agriculture Organization of the United Nations (FAO) or the World Health Organization (WHO). Neither FAO nor WHO is responsible for the content or accuracy of this translation. The original [Language] edition shall be the authoritative edition.

Disputes arising under the licence that cannot be settled amicably will be resolved by mediation and arbitration as described in Article 8 of the licence except as otherwise provided herein. The applicable mediation rules will be the mediation rules of the World Intellectual Property Organization http://www.wipo.int/amc/en/mediation/rules and any arbitration will be conducted in accordance with the Arbitration Rules of the United Nations Commission on International Trade Law (UNCITRAL).

Users wishing to reuse material from this work that is attributed to a third party, such as tables, figures or images, are responsible for determining whether permission is needed for that reuse and for obtaining permission from the copyright holder. The risk of claims resulting from infringement of any third-party-owned component in the work rests solely with the user.

FAO information products are available on the FAO website (www.fao.org/publications) and can be purchased through publications-sales@fao.org. Requests for commercial use should be submitted via: www.fao.org/contact-us/licence-request. Queries regarding rights and licensing should be submitted to: copyright@fao.org.

Cover picture © Dennis Kunkel Microscopy, Inc

Contents

Acknowledgements

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) would like to express their appreciation to all those who contributed to the preparation of this document through the provision of their time, expertise and other relevant information before, during and after the meeting. Special appreciation is extended to all the members of the Expert Panel for their dedication to this project and to Dr Ed Topp for his expert chairing of the Panel. All contributors are listed on the following pages. FAO and WHO also wish to thank the World Organisation for Animal Health (OIE) for cooperation and input before, during and after the Expert meeting.

Appreciation is also extended to all those who responded to the calls for data that were issued by FAO and WHO and provided information that was not readily available in the peer reviewed literature or in the public domain.

FAO and WHO would also like to acknowledge the financial resources provided by Canada and Republic of Korea to support this work.

Contributors

INVITED EXPERTS

Dr Alexandra Adams, Chair and Professor, Aquatic Immunology and Diagnostics, University of Stirling, Stirling, United Kingdom

Dr Priyanie Amerasinghe, Senior Researcher, Human and Environmental Health, International Water Management Institute, Colombo, Sri Lanka

Dr John Paul Brooks, Research Microbiologist, Genetics and Sustainable Agriculture Research, Agricultural Research Service, United States Department of Agriculture, Washington DC, United States of America

Dr Teresa M. Coque, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS)**,** Hospital Universitario Ramón y Cajal, Microbiology Department, Madrid, Spain

Dr Seamus Fanning, Professor, UCD-Centre for Food Safety, School of Public Health, Physiotherapy and Sports Science, University College Dublin, Belfield, Ireland

Dr Ana Maria de Roda Husman, Centre for Zoonoses and Environmental Microbiology, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Dr Saharuetai Jeamsripong, Microbial Food Safety and Antimicrobial Resistance, Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

Dr David Jordan, Principal Research Scientist, New South Wales Department of Primary Industries, Wollongbar, Australia

Mr Amit Khurana, Director**,** Food Safety and Toxins Programme**,** Centre for Science and Environment**,** New Delhi**,** India

Dr Scott McEwen, Professor, Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Canada

Dr Johanna Muurinen, Researcher, Faculty of Agriculture and Forestry, Department of Microbiology, University of Helsinki, Helsinki, Finland

Dr Irene Bueno Padilla, PhD Candidate, University of Minnesota, Minneapolis, United States of America

Dr George Sundin, Professor, Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, United States of America

Dr Ed Topp (Chair), Principal Research Scientist, Agriculture and Agri-Food Canada/University of Western Ontario, London, Canada

Dr Jaap Wagenaar, Professor, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Dr Fiona Walsh, Lecturer, Department of Biology, Maynooth University, Maynooth, Ireland

Dr Kassahun Asmare Wondim, Professor, Deputy Regional Director, Global One Health Initiative of the Ohio State University, Eastern Africa Regional Office, Addis Ababa, Ethiopia

Dr Likou Zou, Professor, College of Resources, Sichuan Agricultural University, Ya'an, China

JOINT SECRETARIAT

Food and Agriculture Organization of the United Nations (FAO)

Dr Alejandro Dorado-Garcia, Food Safety and Antimicrobial Resistance Consultant, Food Safety and Quality Unit, FAO, Rome, Italy

Dr Jeffrey LeJeune, Food Safety and Antimicrobial Resistance Consultant, Food Safety and Quality Unit, FAO, Rome, Italy

Dr Kang Zhou, Associate Professional Officer, Food Safety and Quality Unit, FAO, Rome, Italy

World Health Organization (WHO)

Dr Amina Benyahia, Coordinator *a.i.* Foodborne and Zoonotic Diseases Unit, Department of Food Safety and Zoonoses, WHO, Geneva, Switzerland

Ms Yuki Minato, Project Officer, Department of Food Safety and Zoonoses, WHO, Geneva, Switzerland

Dr Astrid Louise Wester, Medical Officer, Department of Public Health, Environment and Social Determinants of Health, WHO, Geneva, Switzerland

Dr Tomoyuki Yamaguchi, Consultant, Department of Food Safety and Zoonoses, WHO, Geneva, Switzerland

COLLABORATING ORGANIZATION

World Organisation for Animal Health (OIE)

Dr Jorge Pinto Ferreira, Chargé de Mission, Science and New Technologies Department, OIE, Paris, France

RESOURCE PERSONS

Dr Daniela Battaglia, Animal Production Officer, Animal Production and Health Division, FAO, Rome, Italy

Ms Gracia Brisco, Food Standards Officer, Joint FAO/WHO Food Standards Programme (Codex Secretariat), Rome, Italy

Dr Sarah Cahill, Senior Food Standards Officer, Joint FAO/WHO Food Standards Programme (Codex Secretariat), Rome, Italy

Ms Verna Carolissen-Mackay, Food Standards Officer, Joint FAO/WHO Food Standards Programme (Codex Secretariat), Rome, Italy

Ms Myoengsin Choi, Food Standards Officer, Joint FAO/WHO Food Standards Programme (Codex Secretariat), Rome, Italy

Dr Gunilla Eklund, Animal Health Officer, Animal Production and Health Division, FAO, Rome, Italy

Dr Hyo Sun Kwak, Director, Food Microbiology Division, Food Safety Evaluation Department, National Institute of Food and Drug Safety Evaluation, Cheongju, Republic of Korea

Ms Rosa M. Peran i Sala, Senior Advisor, Department of International Affairs, Ministry of Health, Welfare and Sport, The Hague, The Netherlands

Dr Donald Prater, Assistant Commissioner for Food Safety Integration, Office of Foods and Veterinary Medicine, United States Food and Drug Administration, Maryland, United States of America

Ms Saskia Reppin, Associate Professional Officer, Animal Production and Health Division, FAO, Rome, Italy

Dr Andrew Singer, Senior Scientific Officer, NERC Centre for Ecology & Hydrology, Wallingford, United Kingdom

Declarations of interest

All participants completed a Declaration of interests form in advance of their involvement in in this work.

One of the Experts declared an interest in the topic under consideration:

Dr Andrew Singer declared that he owned stocks in various pharmaceutical and vaccine manufacturers. Upon detailed review of the declaration, it was considered that the activities of Dr Andrew Singer represent a potential conflict of interest. Therefore, he was invited to the meeting, but did not participate in the final adoption of the conclusions and recommendations of the meeting.

All of the declarations, together with any updates, were made known and available to all the participants at the beginning of the meeting.

All the Experts participated in their individual capacities and not as representatives of their country, government or organization.

Abbreviations

AMR	Antimicrobial Resistance
AMU	Antimicrobial Use
ARGs	Antimicrobial Resistance Genes
CAC	Codex Alimentarius Commission
ECOFFs	Epidemiological Cut-Off Values
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
IPM	Integrated Pest Management
LMICs	Low- and Middle-Income Countries
MIC	Minimum Inhibitory Concentration
MSC	Minimum Selective Concentration
OIE	World Organisation for Animal Health
RASFF	Rapid Alert System for Food Products
TFAMR	Task Force on Antimicrobial Resistance
WHO	World Health Organization

Executive summary

There is clear scientific evidence that foods of plant origin may serve as vehicles of foodborne exposure to antimicrobial-resistant bacteria. Fruits, vegetables and other foods of plant origin can become contaminated with antimicrobial-resistant bacteria and antibiotic resistance genes (ARGs) anywhere along the food chain, from primary production to consumption. Conventionally and organically grown vegetables to be consumed raw may be vehicles for dissemination of antimicrobial-resistant bacteria and their resistance genes to humans. Concerted efforts should be made to mitigate their contamination at all stages of the food chain, from production to consumption. Important sources of microbial contamination in the pre-harvest environment include soil, organic fertilizers and irrigation water. Hence, good agricultural hygienic practices should be employed during pre-harvest stages of food production.

Use of antimicrobials in humans and animals selects for antimicrobial-resistant bacteria in faeces. Up to more than 80 percent of the antimicrobial administered (as well as copper and zinc from the diet) is excreted in the faeces and urine in an active form. Thus, manure or other organic materials containing human or animal wastes that are used as soil amendments have the potential to disseminate both residues of antimicrobial agents and antimicrobial-resistant bacteria to the environment. Vegetables harvested from manure-treated ground can carry an additional burden of ARGs of enteric or environmental bacterial origin.

Water can also be an important source of antimicrobial residues, antimicrobial-resistant bacteria and ARGs. There is a direct link between water quality used for irrigation and antimicrobial-resistant bacteria on foods. Wastewater effluent recovered from municipal sewage may contain ARGs and antimicrobial-resistant bacteria. Consequently, soils irrigated with wastewater can also become contaminated with ARGs and with multidrug antimicrobial-resistant bacteria. Water found adjacent to manured fields may also be enriched in antimicrobial-resistant bacteria.

Aquaculture products (e.g. fish, shellfish and shrimp) can carry bacteria that are resistant to medically important antimicrobials. Aquaculture primary food production systems that receive antimicrobials, or that are exposed to effluents containing antimicrobial residues and/or faecal material of human or animal origin, can become enriched in antimicrobial-resistant bacteria. Additionally, aquaculture production has the potential to contaminate water used for irrigation. Using water contaminated with this effluent for irrigation purposes provides a direct route of contamination of fruits and vegetables, if such water is applied directly to the edible portions of the plant. Aquaculture systems can vary substantially between countries or regions in ways that may variably impact the risk of acquiring and disseminating antimicrobial resistance (AMR).

Special emphasis should be placed on so-called integrated food production systems. Here, crops are produced together with food of aquaculture origin, based on water contaminated with human or animal waste. This may constitute a resource-efficient system, including from a waste management viewpoint. Chemical disinfectants are critical for food hygiene and environmental sanitation. Bacteria with increased tolerance to biocides have been recovered from food production environments. Although there is theoretical and experimental evidence that certain microbiocidal agents may co-select for AMR, there is an absence of empirical data to indicate that the use of biocides drives this co-selection under the conditions present in the food production or processing environments.

Antimicrobials are vital to treat and control plant diseases. Contamination of soils with these products following crop application leads to enrichment of antimicrobial-resistant bacteria and ARGs in the environment. However, the extent to which the treatment of crops with antimicrobial agents (or copper formulations, see below) promotes AMR in bacteria found on edible portions of fresh plant produce is uncertain.

Of concern is the possibility of selection of antimicrobial-resistant bacteria and ARGs through the processes of co-resistance, cross-resistance and co-regulation with certain metal ions. Contamination of soil with certain metal ions, such as copper ions, can promote AMR in soil bacteria. In addition to copper-containing products being used to treat plant diseases, animal and human wastes often have residue levels of copper, zinc and other metals of dietary origin. Bacteria harbouring genes conferring resistance to certain metal ions (and in some cases to certain biocides) are more likely to also carry ARGs than those without such metal ion resistance traits. Bacteria resistant to both metal ions and antimicrobials are commonly present in diverse environments, with bacteria of plant origin having the highest frequency of resistance to both metals and antimicrobials, compared with bacteria from other sources such as domestic animals, wild animals or humans.

Given the potential of human exposure to antimicrobial-resistant bacteria via foods of plant origin and from aquaculture products, there is considerable value in incorporating these products into integrated antimicrobial use (AMU) and AMR surveillance systems. Although *E. coli* may serve as a suitable common indicator bacterium for antimicrobial-resistant bacteria in foods of animal origin, there is a need to identify additional robust indicators of antimicrobial-resistant bacteria in foods of plant origin and the immediate crop production environment. Likewise, there are no universally accepted bacterial indicators of AMR in aquatic products. AMR surveillance should use culture and antimicrobial susceptibility testing based on epidemiological cut-off values (ECOFFs) and may need to include molecular methods for ARG analysis, and antimicrobial residue chemical analyses. Antimicrobial-resistant bacteria, ARGs and AMU surveillance in fruit and vegetable production systems should capture all important metadata for the antimicrobials, such as information from manufacturers, importers and vendors, where possible.

Conclusions

- Best management practices should be adhered to with respect to the use of faecal material of human (sewage sludge, biosolids) or animal (manures) origin in primary food production environments, as well as the use of reclaimed wastewater for irrigation.
- Improved methods for infection prevention and control such as husbandry, biosecurity, diagnostics, vaccines and other alternatives and adjuvants to antimicrobials should be employed to reduce the need for AMU in aquaculture, and thereby reduce the antimicrobial contamination of the primary aquaculture production environment.
- Biocides should be used according to manufacturers' recommendations.
- Antimicrobials should only be used in crop production according to label guidelines in the context of integrated pest management (IPM) strategies.

Introduction

In recognition of the growing problem of antimicrobial resistance (AMR), its increasing threat to human, animal and plant health, and the need for a One Health approach to address this issue, the 39th Session of the Codex Alimentarius Commission (CAC) agreed it was important for the food safety community to play its part and re-established the ad hoc Codex Intergovernmental Task Force on Antimicrobial Resistance (TFAMR) (CAC, 2016). The objectives of the Task Force were to revise the current *Codex Code of Practice to Minimise and Contain Antimicrobial Resistance* (CAC/RCP 61-2005) (CAC, 2005) and to develop new guidance on surveillance programmes relevant to foodborne AMR.

Responding to the request from the CAC and the Task Force to provide scientific advice in the areas of crops, environment and biocides (CAC, 2018), the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) convened, in collaboration with the World Organisation for Animal Health (OIE), a joint “FAO/WHO expert meeting on foodborne antimicrobial resistance: role of environment, crops and biocides” on 11-15 June 2018 in Rome, Italy.

The primary purpose of the meeting was to synthesize the current scientific literature concerning the transmission of antimicrobial-resistant bacteria, antimicrobial residues and antimicrobial resistance genes (ARGs) from environmental sources (e.g. contaminated water, soil, manure or human wastes, fertilizers, processing and transportation facilities) to foods and feeds of plant and aquatic animal origin.

As a secondary goal, given the widespread and frequent use of disinfectants in food processing plant sanitation, the potential of biocides to co-select for AMR and ARGs was also reviewed. Non-food crops (e.g. cotton, flower bulbs) were excluded from the scope of consideration.

The meeting therefore addressed the following priority areas: the prevalence of antimicrobial-resistant bacteria and ARGs in fruits and vegetables (Section 1); antimicrobial residues, antimicrobial-resistant bacteria and ARGs in the immediate food production environment – namely in soils (Section 2.1), irrigation water (Section 2.2) and aquaculture (Section 2.3); use of antimicrobials and copper in horticulture production (Section 2.4); defining environmental quality thresholds for antimicrobials (Section 2.5); use of biocides in the food processing environment (Section 3); and crops, aquaculture products and their production environments in integrated surveillance of AMR (Section 4).

To answer the request from the CAC and the Task Force, FAO and WHO, in collaboration with OIE, have planned a series of expert consultations to address both the risk assessment aspects and the risk management component of the request for scientific advice. Furthermore, Codex requested that this advice should seek to identify any further issues and specific gaps in current scientific knowledge that need to be considered in the revision of existing Codex texts and/or development of new Codex texts. To ensure transparency, public calls for experts[1] and data[2] were published and disseminated globally. Responses were received and suitable experts selected, after consideration of any declared interests by the experts.

---

[1] http://www.fao.org/fileadmin/user_upload/agns/pdf/Call_for_data_experts/EXPERTS_Foodborne_AMR.pdf or http://www.who.int/foodsafety/Call_for_experts_oct2017.pdf

[2] http://www.fao.org/fileadmin/user_upload/agns/pdf/Call_for_data_experts/DATA_Foodborne_AMR.pdf or http://www.who.int/foodsafety/DATA_Foodborne_AMR.pdf

1

Contamination of crops with antimicrobial-resistant bacteria

The microbiological contamination of foods of plant origin (e.g. fruits, vegetables, lettuce) that are consumed raw or undercooked is responsible for foodborne illnesses worldwide, including outbreaks of disease caused by antimicrobial-resistant bacteria. In addition to antimicrobial-resistant pathogens, produce at retail can also be contaminated with non-pathogenic bacteria that may carry resistance to medically important antimicrobials (Verraes *et al.*, 2013; Thanner *et al.,* 2016).

Bacteria and fungi cause significant plant disease and production losses worldwide, especially in low- and middle-income countries (LMICs). Climate change is predicted to exacerbate this problem and the use of antimicrobial agents is expected to rise concomitantly as older treatments become ineffective and are discontinued and as disease burden continues to climb. There is growing concern that antimicrobials are losing their effectiveness in all sectors – not only in horticulture but also in veterinary and human medicine. Extensive use and misuse of antimicrobials drives the development and transmission of AMR, but it is unclear the extent to which antimicrobial use (AMU) is driving the development of AMR specifically in plant pathogens, soil organisms, spoilage organisms and non-pathogenic contaminants and zoonotic agents present on foods of plant origin.

Some of the same drugs that are used in human and veterinary medicine (e.g. streptomycin, tetracyclines, triazoles) are also used to control plant diseases.

Thus, resistance that develops in one sector can be transferred and clinically relevant across sectors and species. Through processes of co-selection and cross-resistance, resistance that develops to one antimicrobial may also render an organism resistant to several unrelated drugs and chemicals. Bacteria, notably zoonotic organisms with resistance to one or multiple antimicrobials, are found on fruits, vegetables and other edible plants, as well as in soils. The frequency with which resistant bacteria of plant origin colonize the human gastrointestinal tract or serve as reservoirs of AMR genes in the gastrointestinal tract needs to be determined. There is convincing evidence that agricultural AMU is driving the emergence of antimicrobial-resistant fungi that are increasingly transmitted from the environment to humans.

Several antimicrobials are approved for use specifically to treat bacterial plant diseases in at least 20 countries. In countries where regulations and oversight of AMU are strong, the use of antimicrobials and their residues on foods of plant origin is minimal. However, in other countries, the quantities and types of antimicrobials being used for agronomic application are undocumented – a problem compounded by challenges of access to quality-assured antimicrobials, including a growing industry of fraudulent and substandard products. The consequences of AMU in plant production, resulting in occupational exposure, food and environmental contamination, need to be assessed in order to develop science-based recommendations for mitigating the negative public health impacts of AMR.

As fruits and vegetables are frequently eaten raw or with minimal processing, it can be stated that fresh fruits and vegetables serve as a source of dietary exposure to antimicrobial-resistant bacteria and ARGs. Other reports also confirm the role that foods of plant origin play in the foodborne transmission of antimicrobial-resistant bacteria (Bezanson *et al.*, 2008; Boehme *et al.*, 2004; Hassan *et al.*, 2011; Raphael *et al.*, 2011; Rodríguez *et al.*, 2006; Ruimy *et al.*, 2010; Schwaiger *et al.*, 2011; Walia *et al.*, 2013). Therefore, reducing the contamination of foods and feeds of plant origin with antimicrobial-resistant bacteria will reduce human and animal exposure to antimicrobial-resistant bacteria and ARGs.

Resistant bacteria from multiple sources can contaminate foods of plant origin. The soil is replete with bacteria that harbour AMR genes. Direct contact of edible portions of plants with soil and soil splash can contribute to food contamination. Animal and human wastes introduced intentionally as soil amendments or through animal intrusion provide another pathway for antimicrobial-resistant bacteria to contaminate foods of plant origin. Water used for irrigation may also be contaminated with antimicrobial-resistant organisms. Clearly, the adoption of good agricultural practices that limit total microbial contamination of foods of plant origin is a critical first step in reducing the introduction of antimicrobial-resistant organisms into the food chain.

Contributing to the problem of AMR is the fact that there are very few products to treat bacterial infections of plants. The risk of development of resistance in plant pathogen populations is widely understood in horticultural production systems; among bacterial plant pathogens, resistance is reported for products commonly used to treat bacterial diseases (e.g. streptomycin, tetracycline, kasugamycin, copper). With regard to antifungals, resistance to triazole fungicides is also well documented and relatively common, although there is a great deal of variation in the frequency of resistance between regions and among pathogens.

Production practices are available that can minimize AMR risks by reducing the need for AMU. Biological control (introduction of organisms that provide direct antagonism, competition, hyperparasitism or induction of host plant resistance) and biorational products, such as plant extracts, can prevent and treat plant diseases. These products are considered to present lower risks to the environment and human health. However, biological and biorational products are generally far less effective than antimicrobials and their performance is inconsistent over time and across locations. As more is learned about phytobiome functions in food crop systems, more effective pre- and probiotic agents against plant pathogens may be developed to reduce the need for conventional antibacterial and antifungal agents.

By far the most effective approach to limit the use of antimicrobials in plant production is through the use of the well-established procedures of "Integrated Pest Management" (IPM) – a systems approach designed to minimize economic losses for crops, as well as to minimize risks to people and the environment through the use of pesticides. Key components of IPM for preventing and managing plant diseases are:

- **Accurate and timely diagnosis and monitoring**, which can also include disease modelling and predictive systems to optimize timing of plant protection product applications;
- **Use of disease-resistant crop varieties**, including resistant rootstocks in both fruit and vegetable systems;
- **Exclusionary practices** (biosecurity) that prevent the introduction of pathogens into a crop, such as using pathogen-free true seed and vegetative planting material, clean irrigation water and sanitation practices that prevent the movement of pathogens from plant to plant and field to field;
- **Careful site selection and soil improvement** to maximize plant health and minimize environmental factors that favour pathogens;
- **Crop rotation** and other cultural practices to prevent pathogen build-up;
- **Use of biological and biorational products**; and
- **Judicious use of antimicrobials**, including those for the treatment and control of bacterial and fungal diseases.

While many growers in developed countries are aware of and practice disease management strategies, improved uptake of these specific practices, especially in LMICs, will help to reduce infection pressure and consequently the need for antimicrobials. IPM should continue to be emphasized in grower and gardener education programmes in developed economies and should be widely encouraged through governmental and non-governmental programmes in LMICs. The importance of IPM for slowing the development of AMR and promoting food security and human and animal health cannot be overstated.

Additional information, tools and activities are urgently needed to better understand and mitigate the risks associated with AMR from agronomic sources, especially in LMICs. For example, advances in surveillance, good practices, awareness and strengthened government regulation and oversight for AMU and surveillance will contribute to a more effective One Health approach to combat AMR.

The largest barrier to understanding the role of plant-based agriculture in the holistic picture of AMR ecology is the lack of relevant data. Data on the dissemination of antimicrobial-resistant organisms from crops that are consumed raw and their impacts on human health are sparse. Information is particularly lacking for LMICs. Systems to record AMU and antimicrobial-resistant organisms on fruits and vegetables at the national level are virtually absent. Surveillance systems for foods of plant origin should be developed in such a way that they can be integrated and harmonized with surveillance in other sectors, including AMR programmes in humans, animals and foods of animal origin, to better assess risks and priority areas for intervention. In addition to AMR among plant pathogens, it is important to monitor animal, human and zoonotic pathogens on plants as well as the resistome of other organisms in the plant production environment, which may also contribute resistance genes to the food chain. The creation of new, rapid and inexpensive tests and tools to diagnose plant diseases and characterize the resistome of the plant production environment will help to establish more appropriate surveillance strategies and AMU guidelines. To this end there are challenges that need to be addressed in developing these surveillance programmes. One key challenge will be determining an appropriate standard denominator to characterize AMU (e.g. kilograms of oxytetracycline used per tonne of dates or apples produced) so that trends within and across countries can be monitored in kind.

Few methods are available to reduce or eliminate bacteria or AMR genes from fruits and vegetables that are consumed raw or with minimal processing. Therefore, prevention of contamination at all stages of production and processing is paramount to minimize the introduction of antimicrobial-resistant organisms into the food chain from plant-based foods. Development, validation and application of additional contamination prevention strategies along the entire food chain could greatly reduce antimicrobial-resistant organisms and ARGs in foods of plant origin.

Due to the limited number of medicines available to effectively treat plant diseases, additional strategies to prevent, control and treat plant diseases need to be developed, especially interventions and products with systemic effects. Examples of valuable innovations may include the following:

- **Selective breeding** to decrease host plant susceptibility to diseases or to enable plants to degrade antimicrobials to reduce soil contamination;
- **Discovery/development of drugs with antifungal and/or antibacterial activity** with different modes of action, not shared with drugs used in human medicine;
- Use of **effective biologicals** (probiotics, prebiotics, bacteriophages) **and biorational compounds** for disease control;
- **Exploitation of the microbiome and soil health** to control plant diseases; and
- More effective **integrated disease and pest management strategies**.

Additional specific information is needed to quantify the relationship between the use of antimicrobials, other plant protection products, and other influences on the selection, transmission and persistence of AMR among organisms on plants and in the surrounding food production environment.

A paradigm shift in plant-based food production practices, and acceptance of these practices by producers, are needed to reduce AMU. Awareness of the severity of the problem and adoption of sustainable solution pathways at all stages in the food chain are critical to slow the development of AMR and mitigate its negative consequences. For food producers, this means recognizing that AMR can contribute to production and economic losses at all scales of production. AMR can also cause direct and serious health impacts on producers who apply antimicrobials, and on their families and customers who consume products contaminated with antimicrobial residues and antimicrobial-resistant bacteria (Marshall and Levy, 2011). In some countries, buyers are demanding commodities produced with strong antimicrobial stewardship practices, such as treating only after a correct diagnosis, appropriate application and dosing, respecting pre-harvest intervals, and incorporating IPM practices. Lack of understanding and incentives for producers to employ better practices remains an obstacle to effective management of AMR and this problem is further complicated by misinformation and availability of products on the market that are fraudulent, substandard or otherwise lacking evidence of effectiveness.

1.1 CROPS FOR HUMAN CONSUMPTION

Human and animal waste is frequently used as a valued fertilizer in crop production. However, if excreta are not treated properly, they may carry antimicrobial-resistant pathogens (Marshall and Levy, 2011). If plant food products that are consumed raw or undercooked are contaminated through environmental sources, they will be collectively contaminated with a whole range of bacteria and with resistance and virulence genes of human, animal and environmental origin. Other sources of contamination include water, air and soil.

1.2 CROPS FOR ANIMAL CONSUMPTION

Terrestrial animals (e.g. ruminants) forage on pasture or are fed grains and grass crops (e.g. silage or haylage). It is common practice in production animal farms worldwide that the animal manure is used as a fertilizer for the crops that are grown for forage by the animals. Wildlife, pest animals and insects commonly inhabit farm settings and are known to be able to disseminate bacteria to and from production animals. The forage and feed crops may be contaminated with animal waste, including from both husbandry animals and wildlife (Fenlon, 1985; Nightingale *et al*., 2004). Crops grown for animal feeds may also be contaminated with bacteria from the soil (Heyndrickx, 2011), which commonly harbour ARGs (Wright, 2010). If antimicrobials are administered to production animals while the animals are consuming crops contaminated with antimicrobial resistant bacteria, selection for this population in the animal gut might occur. This could be a route of introduction and amplification of ARGs of environmental origin into the food chain (Marshall and Levy, 2011; Witte, 2000) and should be taken into account when considering whether the animal feed and agricultural soils should be a part of the surveillance. Also, if practices in the production animal farms are modified in ways that affect the frequency of contamination of livestock feeds, new and emerging unpredictable issues might arise. Such practices might be, for example, the use of recycled fertilizers that might harbour contaminants that are not present in manure. In addition to manure and faeces, wildlife, insects and pest animals are suspected to be significant contributors in disseminating AMR between production animals and the environment (Surette and Wright, 2017); however, understanding their role and the possible risks would require systematic investigations. For example, research from Norway indicates that the prevalence of AMR in *E. coli* from red foxes was higher in areas with higher human population density and in areas close to the larger cities than in less populated areas (Norwegian Veterinary Institute, 2017).

2

Antimicrobial-resistant bacteria and ARGs in the immediate plant production environment

Fruits, vegetables and other foods of plant origin can become contaminated with antimicrobial-resistant bacteria and ARGs anywhere along the food chain, from primary production to consumption. Conventionally and organically grown vegetables to be consumed raw may be vehicles of dissemination of antimicrobial-resistant bacteria and their ARGs to humans (Zarfel *et al.*, 2013). Important sources of microbial contamination in the pre-harvest environment include soil, organic fertilizers and irrigation water.

Antimicrobials are widely used for people, livestock, poultry, aquaculture, apiculture, pets and plants, not only for treatment of infections, but also for disease control, prophylaxis and, in some countries, for growth promotion in food-producing animals. Depending on the species treated and the particular drug used, the percentage of the dosage that is absorbed or metabolized by an individual animal or person ranges from as little as 10 percent to over 80 percent, with the remainder excreted as active compounds through urine and faeces into the environment. Soils are contaminated by antimicrobials used for disease control in plant production and by residues in manures and wastes applied as crop fertilizers. Waste streams from humans and animals treated with antimicrobials are also enriched with resistant microorganisms and ARGs.

Persistence dynamics of antimicrobial residues, ARGs and the survival of antimicrobial-resistant organisms in the environment are complex. A number of biotic and abiotic factors, including temperature, solar radiation exposure, pH, soil type and microbial biodiversity, influence how long residues remain in the environment and at what rates bacteria proliferate, die off, exchange resistance genes and are dispersed. Of growing concern are the effects that low concentrations of antimicrobials (lower than minimal inhibitory concentrations) have as a selective force in AMR emergence. Cross-resistance and co-selection can further contribute to the multi-drug resistance problem. Thus, resistance that develops in the environment may be clinically relevant across all sectors.

Many studies have documented antimicrobial residues in ecosystems influenced by both urban and agricultural activities. Likewise, ARGs and bacteria (notably zoonotic organisms) with resistance to one or more antimicrobials can be detected in surface waters, in soils, in animal feeds and on edible plants around the globe. In some regions, guidelines and regulations have been introduced to limit environmental contamination by industrial, human and animal wastes. However, there are still many gaps in knowledge about the ecology of AMR when it comes to environmental contamination with antimicrobial residues, resistant bacteria and ARGs. For example, the magnitude of the public health threat posed by antimicrobial-resistant organisms (and ARGs) in the environment, and the effects of antimicrobial residues on soil ecosystem services, such as biogeochemical cycles, are still unknown.

There are multiple potential sources of antimicrobials entering the environment. Among the most important anthropogenic contributors to environmental pollution with antimicrobials are wastes from pharmaceutical manufacturing, hospitals, wastewater treatment facilities, untreated human wastes, waste and runoff from aquaculture, livestock, and plant-based food production and processing facilities. However, the fraction attributable to each source and the factors governing abundance and distribution of antimicrobial-resistant organisms, ARGs and residues in the environment from each source are unclear. Despite current knowledge gaps, there are several practical and immediate actions that can be taken to minimize environmental contamination with antimicrobial residues, antimicrobial-resistant organisms and ARGs.

- With respect to agricultural sources, reducing the need for AMU through improved animal health and hygiene practices is the single most effective way to proactively reduce the contamination of animal wastes with antimicrobial residues and antimicrobial-resistant bacteria. Biosecurity, vaccination, minimal stress and good feed at the herd or flock level are widely encouraged for many reasons and can be effective for preventing exposure of the herd or flock to antimicrobial-resistant organisms. Actions that target the transmission of specific animal diseases, particularly those caused by bacterial pathogens, are likely to be effective for preventing AMR in the herd or flock.

- Animals may be colonized with antimicrobial-resistant organisms and this can result in a number of environmental niches being contaminated. Where animal production is itself a source of AMR for other food-producing systems (including crop enterprises on the same farm), the biosecurity programme must include strategies for containing AMR. This could include considering/altering the treatment of effluents, restricting the use of farm wastes, and ensuring that commodities leaving the farm are less likely to contain antimicrobial resistant organisms. Managers should assess the risks related to AMR exposure, release and transmission through the environment and integrate appropriate mitigation steps into biosecurity practices.
- Waste treatment protocols vary in their efficacy to remove or decrease antimicrobial residues, depending on the treatment process and the specific antimicrobial in question. Thus, existing waste treatment protocols should be reviewed to assess their efficacy against AMR and adjusted as indicated by the findings. In LMICs, waste treatment facilities and standard operating procedures may be limited or absent. In these circumstances, awareness needs to be generated regarding the importance of AMR to human health, animal health and food production so that resources, infrastructure and effective regulatory action can be directed at the issue.
- Protecting water from contamination with residues is the first step in reducing their impact on the environment. This may be achieved by regulating and enforcing the amount of antimicrobial residues discharged into the environment.

While many developed countries are aware of and practice various levels of environmental protection, more widespread and rigorous implementation of these practices, specifically aimed at reducing antimicrobial residue pollution in the environment, will contribute towards slowing the development of AMR – a priority for all countries in the context of the Global Action Plan on AMR.

Additional information, tools, and activities are urgently needed to better characterize and mitigate the risks associated with antimicrobial residues and antimicrobial-resistant bacteria from agronomic sources in the environment, particularly in

LMICs. Paramount among research priorities is determining the magnitude of the direct and indirect public health costs posed by environmental contamination with antimicrobial residues, antimicrobial-resistant organisms and ARGs. Of comparable urgency is the need to determine the relative fraction of contamination attributable to the various potential sources of environmental antimicrobial residues so that interventions and resource allocation can be prioritized for maximum impact and return on investment.

Additional information is also needed to better understand the impact, effectiveness, costs and benefits of different waste treatment practices, such as composting and manure storage, biochar formation, anaerobic digestion, ozone and ultraviolet light treatment, among others. The role of wildlife as vectors for disseminating AMR and ARGs across sectors warrants study as well. Key variables to measure in future studies include: the interactive effects of the environmental matrix and conditions; the specific microorganisms of interest for the antimicrobial under study; persistence of antimicrobial residues, antimicrobial-resistant organisms and ARGs; and the impact of soil resistome (i.e. collection of ARGs) composition on ecosystem services in general. The latter may be especially relevant for food productivity and safety.

2.1 SOIL

Livestock and humans that have received antimicrobials excrete active antimicrobial residues and bacteria carrying ARGs (Liu *et al.*, 2016; Pope *et al.*, 2009; Zhu *et al.*, 2013). Thus, manure or other organic material containing human or animal wastes that are used as soil amendments, as practiced worldwide, have the potential to disseminate both residues of antimicrobial agents and antimicrobial-resistant bacteria into the environment (Jechalke *et al.*, 2013; Marti *et al.*, 2013; Marti *et al.*, 2014; Muurinen *et al.*, 2017; Pourcher *et al.*, 2014; Rahube *et al.*, 2014; Zhou *et al.*, 2017a; Joy *et al.*, 2013; Xie *et al*, 2018). The fate of these antimicrobial-resistant bacteria, ARGs and antimicrobial residues following application of soil amendments will vary with environmental conditions; for example, the selective properties of the antimicrobial residues can last for weeks to months, and possibly more than a single growing season in humid-temperate regions (Marti *et al.,* 2014; Chen *et al.*, 2018).

2.2 IRRIGATION WATER

Water can also be an important source of antimicrobial residues, antimicrobial-resistant bacteria and ARGs, if the irrigation water (or the soil) comes in contact with the plant part that is consumed (Palacios *et al.,* 2017; Pan and Chu, 2018). There is a direct link between water quality used for irrigation and antimicrobial-resistant bacteria on foods. Wastewater effluent recovered from municipal sewage may contain ARGs and antimicrobial-resistant bacteria (Berendonk *et al.*, 2015; Christou *et al.*, 2017; Karkman *et al.*, 2018; LaPara *et al.*, 2011) and may contaminate irrigation water. Water found adjacent to manure-treated fields may also be enriched in antimicrobial-resistant bacteria (Coleman *et al.,* 2013; Pruden *et al.*, 2006). Comparison of fresh produce and its agricultural environment indicates that the Enterobacteriaceae population found on fresh produce is a reflection of that present in the soil in which it was grown (Blaak *et al.,* 2015). A high degree of genetic relatedness between *E.coli* from irrigation water and lettuce has indicated a possible common waterborne pathway of transmission (Aijuka *et al.*, 2015; Njage and Buys, 2015).

In a Brazilian study, forage maize and tanner grass irrigated with treated wastewater presented high levels of surface contamination with *E. coli* and *Salmonella* spp. (Bevilacqua *et al.*, 2014). Antimicrobial-resistant strains of *E. coli* present in irrigation water and vegetables from 16 household farms were evaluated (Araujo *et al.*, 2017). The same sequence types and indistinguishable clones (as shown by repetitive sequence-based PCR typing) were detected in water and vegetables, suggesting cross-contamination. In a national soil survey, Northeast China was found to be a “hot spot” of ARGs, likely due to long-term wastewater irrigation in the area (Zhou *et al.*, 2017b). The presence of *E. coli* isolates from irrigation water and leafy green vegetables in different food production systems (large commercial farms, small-scale farms and homestead gardens) was investigated (Jongman and Korsten, 2016). In that study, the prevalence of multidrug-resistant *E. coli* was lower in isolates from farms certified as implementing specific good agriculture practices to prevent contamination (Global GAP-certified) than among isolates from non-certified commercial and small-scale farms and homestead gardens. An *E. coli* transmission link between the irrigation water sources and leafy green vegetables was established using both phenotypic (AMR) and genotypic (DNA fingerprinting) analyses.

Constructed wetlands are used as biological treatment of animal, human and industrial waste. Their efficiency with regard to the removal of antibiotics and ARGs varies according to type of antibiotic and ARG (Chen *et al.*, 2016). In some locales, however, such wetlands are concomitantly used for food production (i.e. crops and/or food of aquatic origin). New evidence indicates that these integrated wetland food production systems may be implicated in AMR spread (Krzeminski *et al.*, 2019).

2.3 AQUACULTURE

In 2014, with production of 73.7 million tonnes of aquatic animals (including marine and freshwater finfish, crustaceans and shellfish) with a value of USD 130 billion, the contribution of aquaculture to supply food for human consumption overtook that for wild-caught fish for the first time (FAO, 2016a). In 2016, this production increased to 80.0 million tonnes (FAO, 2018). Diseases are still considered to be a major constraint to aquaculture globally. It has been estimated that 10 percent of all cultured aquatic animals are lost because of infectious diseases alone, amounting to >10 billion USD in losses annually on a global scale (Evensen, 2016). Antimicrobials are routinely used in aquaculture for the treatment of bacterial diseases (FAO, 2016b). Although antimicrobials are effective in aquaculture, there are concerns about AMU (abuse, overuse, misuse) with regard to human, animal and ecosystem health issues related to the development and dissemination of antimicrobial-resistant organisms through the environment (FAO/OIE/WHO, 2006).

Aquaculture products (e.g. fish, shellfish and shrimp) at retail can carry bacteria that are resistant to medically important antimicrobials (Elbashir *et al.*, 2018; Done *et al.*, 2015). Data indicate that aquaculture primary food production systems that receive antimicrobials, or that are exposed to effluents containing antimicrobial residues or faecal material of human or animal origin, can become enriched in antimicrobial-resistant bacteria (Novais *et al.*, 2018). Additionally, aquaculture production has the potential to contaminate water used downstream for irrigation (Done *et al.,* 2015*).* Using water contaminated with this effluent for irrigation purposes provides a direct route for contamination of fruits and vegetables, if such water is applied directly to the edible portions of the plant (Watts *et al.*, 2017; Cabello *et al.*, 2013; Tendencia *et al.*, 2001; Miranda and Zemelman, 2002; Jang *et al.*, 2018).

The diversity of production systems (intensification, size, location, species, marine vs freshwater, etc.) needs to be considered when assessing the risk of AMR. Differences between aquaculture systems are remarkable among countries and may variably impact the risk of acquiring and disseminating AMR (FAO/WHO, 2003). The pathways for introduction and transmission of antimicrobial-resistant bacteria in aquaculture production systems include:

- Integrated food animal production systems (e.g. poultry and fish) are common in small-scale aquaculture. There is evidence for development of AMR in these systems (Petersen *et al.*, 2002; Cabello *et al.*, 2016).
- Manure from swine and poultry production systems is used as feed or feed supplement in some aquaculture production systems (e.g. pond-raised tilapia) (Minich *et al.*, 2018; Elsaidy *et al.*, 2015).

- Run-off water potentially contaminated with human or animal waste can be directed into some fish ponds in some parts of the world to maintain water levels.
- There is a potential risk of foods of aquatic origin being contaminated with AMR organisms both pre-harvest (water, sediment, effluent from the farms) and post-harvest (processing, packaging, storage) (Singh *et al.*, 2016).
- Sediment from retention ponds (i.e. lagoons) from some aquaculture production systems (e.g. freshwater rainbow trout in Chile) is used as fertilizer in horticulture (e.g. berry production).

Antimicrobial-resistant organisms are common in the aquaculture environment, but there is a need for robust scientific evidence to assess the association between AMU and increased prevalence of antimicrobial-resistant organisms in the environment and in foods of aquatic animal origin, as well as their potential human health implications. It is also important to understand secular and seasonal trends in AMR in the aquaculture environment. More evidence about the pathways of AMR dissemination from aquaculture farms is needed, as there is potential risk entering the food chain (sediment used as fertilizer, for example; Wellington *et al.*, 2013; Singer *et al.*, 2016; Bueno *et al.*, 2018). Collection of data on antimicrobial usage in aquaculture globally is needed; standardized methods for measuring AMR in aquatic species pathogens are also needed (Stärk *et al.*, 2018; Ngo *et al.*, 2018; Adams *et al.*, 2011). Co-selection for resistance may occur, but the extent to which this contributes to AMR in aquatic animal production environments needs further investigation (Fernández-Alarcón *et al.*, 2010; He *et al.*, 2017).

2.4 USE OF ANTIMICROBIALS AND COPPER IN HORTICULTURE PRODUCTION

Antimicrobials, including gentamicin, streptomycin, kasugamycin, oxytetracycline, and oxolinic acid, are vital to treat and control plant diseases (de León *et al.*, 2008; Stockwell and Duffy, 2012). Of these, streptomycin and oxytetracycline are approved for horticultural use in several countries. In the United States and New Zealand, antimicrobials (streptomycin, oxytetracycline, kasugamycin) are mostly used for the management of fire blight disease in apple and pear, with usage strictly regulated according to the chemical label. In these situations, antimicrobial applications are typically limited to bloom time, approximately 4-5 months prior to harvest. However, in many other countries, AMU to treat plant disease is unregulated and unmonitored. In these situations, the identity of the crop, disease targeted, spray timings, rates, frequency and time to harvest are unknown.

Contamination of soils with these products following crop application leads to enrichment of antimicrobial-resistant bacteria and ARGs in the environment (Singer *et al.,* 2016). However, the extent to which the treatment of crops with antimicrobial agents (or copper formulations; see below) promotes AMR in bacteria found on edible portions of fresh plant produce is uncertain (Thanner *et al.,* 2016*).* While usage information including total amounts used and crops treated is available from countries such as New Zealand and the United States, such information is not readily available from most countries (McManus, *et al.*, 2002; MPI, 2016).

Widespread use of streptomycin in horticulture, which began in many countries in the 1950s, was followed by the detection of streptomycin resistance in target plant pathogens, including *Erwinia amylovora*, *Pseudomonas syringae* and *Xanthomonas campestris,* as early as the 1970s. This resistance is mediated by chromosomal mutations and plasmid-encoded genes. The most commonly described mechanism of streptomycin resistance is the acquisition of *strAB* genes, which in many cases are located on Tn*5393* (Sundin and Wang, 2018). Despite their widespread use, resistance to kasugamycin, oxytetracycline and oxolinic acid in plant pathogens is less frequent (Sundin and Wang, 2018). It can be difficult to conclude that AMU is enriching the environmental reservoir of resistance because genes responsible for tetracycline and aminoglycoside resistance are naturally detected in bacteria isolated from soil and would likely be detected in most terrestrial habitats (Agga *et al.*, 2015; Versluis *et al.*, 2015). In some cases, screening of non-target bacteria isolated from orchards sprayed with streptomycin revealed the presence of *strAB* genes and, in some cases, transposon Tn*5393* (Norelli *et al.*, 1991; Sobiczewski *et al.*, 1991; Chiou and Jones, 1995; Sundin *et al.*, 1995). In addition, several tetracycline-resistance genes, including *tetA*, *tetB*, *tetC* and *tetG*, were present in tetracycline-resistant epiphytic bacteria in two apple orchards in Michigan USA with no or limited exposure to oxytetracycline (Schnabel and Jones, 1999); however, tetracycline resistance has yet to be reported in the *E. amylovora* apple pathogen. Importantly, it should be noted that surveillance for AMR among phytopathogens is not global in scope, nor are samples often collected at time of harvest when the risks for food contamination would be most significant.

In contrast to AMU, copper-based bactericides are very commonly used (overall several orders of magnitude above antimicrobials) on a wider variety of crops, likely in most countries of the world as these compounds represent the sole bactericide available on many crops. Copper use also typically involves multiple spray applications per season. Copper resistance is widespread in plant pathogenic bacteria isolated from many continents and typically is plasmid-encoded (Lamichhane *et al.*, 2018). Of particular concern is the possibility of selection of antimicrobial-resistant bacteria and ARGs through the processes of co-resistance, cross-resistance and co-regulation with certain metal ions (Yu *et al.*, 2017). Evidence indicates that contamination of soil with certain metal ions, such as copper ions, promotes AMR in soil bacteria. Not only are copper-containing products used to treat plant diseases, but animal and human wastes often have residue levels of copper, zinc and other metals of dietary or industrial origin (Berg *et al.*, 2010). Pal *et al.* (2015) conducted an extensive analysis of the associations between antimicrobial and metal resistance genes. Using a pollution-induced tolerance approach, soils historically (>80 years) contaminated with copper were found to have an increased level of resistance to antimicrobials (e.g. vancomycin), implying that copper represents a strong pressure for co-selection of AMR (Berg *et al.*, 2010).

Co-resistance can occur when the genes for resistance to antimicrobials and to metals are both present in a bacterium, as found in approximately 5 percent of bacterial isolates recovered from plants and soils (Pal *et al.*, 2015). Bacteria harbouring genes conferring resistance to certain metal ions, and in some cases to certain biocides, are more likely to also encode ARGs than those without such metal or biocide resistance traits (Pal *et al.,* 2015). Bacteria resistant to both metal ions and antimicrobials are commonly present in diverse environments, with bacteria of plant origin having the highest relative abundance of co-resistance genes per genome, compared to bacteria from other sources, such as domestic or wild animals and humans (Pal *et al.*, 2015). In the aforementioned study (Pal *et al.*, 2015), none of the bacterial isolates of plant or soil origin analyzed harboured genes for AMR and metal tolerance on the same plasmid, thus suggesting a limited significance of co-selection by metal for the horizontal gene transfer of AMR plasmids.

Nevertheless, plasmids co-encoding for metal resistance and AMR have been identified in bacteria isolated from humans and animals (Bennett, 2008; Argudín *et al.*, 2017). Feeding of copper sulfate to pigs is widely practised for health and growth promotion purposes. However, doing so may select for *Enterococcus faecium* populations resistant to macrolides and glycopeptides (Hasman *et al.,* 2006*).* For example, in *E. faecium*, copper resistance (*tcr*), macrolide resistance (*ermB*) and glycopeptide resistance (*vanA*) are all encoded on a common plasmid. In other studies with Enterococci, co-transfer of copper tolerance (associated with *tcrB*, *cueO*, or an unknown mechanism) and erythromycin, tetracycline, vancomycin, aminoglycosides or ampicillin resistance was demonstrated, indicating genetic linkage between copper tolerance and resistance to several classes of antimicrobials (Silveira *et al.*, 2014). In another example involving a foodborne pathogen, an emerging clinically important clone of *Salmonella enterica* 4,[5],12:i:- with co-resistance to copper and multiple antimicrobials, is circulating in Spain and Southern Europe (Mourão *et al.*, 2015), a region where copper is used extensively in both horticulture and animal agriculture. Copper is known to co-select ARGs including *ermB* and *vanA*, during use in animal agriculture (Seiler and Berendonk, 2012; Pool, 2017). Finally, in one example from plant production, co-resistance to both copper and streptomycin was identified in *P. syringae* strains exposed to both compounds (Sundin *et al.*, 1993). Since manure and sludge are used as fertilizer in horticulture, the use of copper for plant protection could select for AMR among enteric bacteria present in these soil amendments.

2.5 DEFINING ENVIRONMENTAL QUALITY THRESHOLDS FOR ANTIMICROBIALS

2.5.1 Minimum selective concentrations

Management targets related to the crop and environment would ideally be set at concentrations that are below the lowest concentration that allows antimicrobials to select for ARGs. Currently, the best estimate of where this might lie for each antimicrobial is based on experimentally defined and modelled values (Andersson and Hughes 2012, 2014; Bengtsson-Palme and Larsson 2016; Gullberg *et al.* 2011, 2014; Hughes and Andersson 2012; Khan *et al.* 2017; Kraupner *et al.* 2018; Liu *et al.* 2011; Mezger *et al.* 2015; Murray *et al.* 2018; Sandegren 2014; Strukova *et al.* 2016). The experimentally determined estimates of these thresholds are called minimum selective concentrations (MSCs). The MSC is the lowest concentration of antimicrobial at which resistance is positively selected, whereas the minimum inhibitory concentration (MIC), a term commonly used in describing AMR, is the lowest concentration of antimicrobial at which cell growth is visibly inhibited. The MSC can be significantly lower than the MIC (Sandegren 2014). Modelled estimates of an MSC can be found in Bengtsson-Palme and Larsson (2016). The authors derive MSCs from species sensitivity distributions populated with data from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) database. The authors selected the concentration of each antimicrobial representing the 1 percent potentially effective fraction, to which a safety factor of 10 was added to account for the observation that experimentally derived thresholds tend to be approximately an order of magnitude lower than the MIC, while also offering an added level of protection to the estimate. The 111 antimicrobials thresholds ranged from 0.008 µg/L to 64 µg/L. Among the more persistent and ubiquitous classes of antimicrobials are the macrolides and fluoroquinolones. The modelled MSCs for these can be found in Table 1.

TABLE 1. Example modelled minimum selection concentrations for macrolides and quinolones (Bengtsson-Palme and Larsson, 2016)

Antimicrobial class	Drug	Bengtsson-Palme and Larsson (2016)		AMR-Alliance (2018)
		Modelled (µg/L)	Observed Lowest MIC (µg/L) in database	Environmental predicted no-effect concentration-(µg/L)
Quinolones	Ciprofloxacin	0.064	2	0.45
	Levofloxacin	0.25	4	Testing ongoing
	Moxifloxacin	0.125	2	N/A
	Norfloxacin	0.5	16	120
	Ofloxacin	0.5	8	10
Macrolides	Azithromycin	0.25	16	0.02
	Clarithromycin	0.25	8	0.08
	Erythromycin	1	16	0.50

The evidence base for establishing MSC targets for mitigation is currently poor. If the MSCs are obtained from competition experiments between two closely related strains, reliability is likely to be limited when extended to more complex microbial communities (Bengtsson-Palme *et al.*, 2014). The body of research needed to inform such targets will likely take a long time to accumulate. In the interim, it is prudent to use modelled MSC estimates.

2.5.2 Co-selection

When predicting drivers for resistance emergence under environmental conditions, it is important to account for the co-selection mechanisms, such as co-resistance and cross-resistance (Baker-Austin *et al.*, 2006; Di Cesare *et al.*, 2016; Pal *et al.*, 2015; Seiler and Berendonk, 2012; Zhao *et al.*, 2017). Co-resistance refers to different resistance genes present on the same genetic element (e.g. plasmid, transposon, integron), while cross-resistance refers to the same gene conferring resistance to multiple chemicals (e.g. multi-drug efflux pumps), which can be enriched for by a wide range of antimicrobials, biocides and metals. Unfortunately, there is currently no mechanism for estimating the impact from complex mixtures of co-selecting pollutants present in the environment on AMR selection thresholds. However, it is widely assumed that the threshold estimates are not likely to increase, but might further decline (Gullberg *et al.*, 2014). The concept of MSC should apply equally to biocides and metals; however, no published data currently exist.

3

Biocides in food production and AMR

Biocides, notably those used for premise and equipment disinfection and sanitation, are of critical importance for food safety to control microbial cross-contamination and ensure general hygiene at many stages of the food value chain. Active ingredients in biocidal products include a diverse collection of chemicals that may exert a microbiocidal or microbiostatic impact through a range of different mechanisms targeting a broad spectrum of microorganisms. Tolerance to biocidal products may be mediated through transient changes in genotypes and phenotypes (e.g. up-regulation of endogenous genes, mutations). Some individual stressors may also co-select for resistance to multiple classes of antimicrobial drugs because of shared resistance mechanisms (cross-resistance) or genetic linkages (co-resistance) among resistance genes. If cross-resistance to antimicrobials or co-selection of ARGs is driven by routine biocide use, these unintended consequences need to be evaluated and considered by relevant stakeholders (i.e. manufacturers and users) and appropriately managed (Webber *et al.*, 2015).

Currently, biocide use is being questioned due to the possibility that exposure could select for resistance to different antimicrobials (e.g. biocides, heavy metals, antimicrobials) and the induction of lateral gene transfer (Wales and Davies, 2015). Moreover, the presence of biocide residues in the food production environment where antimicrobials are used can facilitate horizontal gene transfer (Verraes *et al.*, 2013). There is ample theoretical and experimental evidence that certain biocide agents may co-select for AMR. For example, increased tolerance to biocide compounds in a few bacterial species of relevance in health and food processing environments has been recently documented (Hardy *et al.*, 2018). Laboratory experiments have demonstrated that interaction between biocides and antimicrobials can influence the development of either tolerance/resistance or collateral sensitivity to different compounds (Curiao *et al.*, 2016; Peter *et al.*, 2018; Oxaran *et al.*, 2018).

However, evidence that biocides select for AMR is based on *in vitro* experiments following guidelines designed for testing antimicrobial susceptibility, using planktonic bacteria (not biofilms) exposed to biocides in aqueous solution either with or without the addition of other co-solvents such as ethanol or dimethyl sulfoxide – conditions that do not mimic the natural environment (Bas *et al.,* 2017; CLSI, 2015). Examples include the use of chlorhexidine resulting in colistin resistance, or triclosan inducing isoniazid resistance. Only a few relevant studies (Romero *et al.*, 2017) reported some data about the co-tolerance, and more data will be needed to address the real situation in the field.

One factor potentially contributing to the development of resistance between biocides, and potentially cross-resistance to antimicrobials, is the use of biocidal agents that rely on a narrow mode of action – i.e. acting on only one or a few bacterial targets. By contrast, resistance to biocides acting on multiple bacterial metabolic pathways would require the simultaneous acquisition of multiple resistance mechanisms to permit survival of the microorganism; a process that is less likely to occur than the acquisition of a single resistance mechanism. While biocides are critical tools for hygiene and food safety, they have the potential to co-select for AMR; therefore, stakeholders need to be made aware of this risk to have the opportunity to conduct risk assessments and implement appropriate strategies to minimize its occurrence.

As more information becomes available to fill research gaps, more specific practices to measure and mitigate the effects of biocide use on AMR development can be promoted. Further research is needed to characterize the potential risks associated with cross-resistance to antimicrobials due to biocide use. Priority data gaps to address include:

- Studies *in situ* under realistic conditions with biocide products and key reference microorganisms or relevant commensals. Biocides are normally formulated with surfactants, sequestrants and other disparate compounds. The few available reports using formulations containing surfactants and sequestrants to base assessments of the risk of induction of changes in bacterial susceptibility are not conclusive (Forbes *et al.,* 2016*).*

- Investigations of the origin of antimicrobial-resistant bacteria found in the food value chain to determine the fraction, if any, attributable to the use of biocides.
- Standardized methods to measure and monitor biocide resistance.

Despite current gaps in knowledge, immediate action can be taken to mitigate potential risks by providing clear guidance to manufacturers and users of biocidal products on practices aimed at minimizing the potential development of resistance. For instance, the appropriate use of biocides in keeping with the manufacturer's instructions and the intended product use, and validation of effectiveness specific to the application, are important for slowing the development of resistance. Improper or excessive use of biocides in the entire food production chain should be avoided as it may potentiate the problem of AMR emergence. Examples of improper use are dilution below the working concentration and using biocide products outside their intended and validated application area. Manufacturers should provide clear instructions to users in this regard and stakeholders would benefit from education and awareness campaigns on proper usage.

Some biocides leave residues that are subsequently discharged into waste streams or otherwise contaminate the environment where they could select for AMR. Therefore, disinfectants that remain active even when they are washed away from food products may be less appropriate for use as biocides in food production and processing because of this potential for widespread impact on the environment and the potential emergence of resistance. Use of technologies to inactivate residual disinfectants, before introducing them into wastewater streams, may be beneficial (Verraes *et al.*, 2013; Barancheshme and Munir, 2017).

Manufacturers may contribute to minimizing the likelihood of AMR development by careful selection of active agents and formulations that target multiple bacterial sites and modes of action less likely to confer cross-resistance. In designing new biocide products, it would be prudent for manufacturers to investigate whether cross-resistance to clinically important antimicrobials is likely to occur under conditions of prescribed use. Conversely, users of biocides could be empowered and enabled to consider monitoring the potential occurrence of cross-resistance in their operations and investigate, where possible, causal links to biocide use or other triggers.

4

Integrated surveillance of AMR and AMU in crops, aquaculture products and their production environments

Given the potential for human exposure to antimicrobial-resistant bacteria via foods of plant origin (Sundin and Wang, 2018) and from aquaculture products (Elbashir *et al.,* 2018; Done *et al.,* 2015), programmes for surveillance of AMU and AMR need to incorporate these food commodities and production systems. As well as sampling the food items themselves, specimens can be collected in the immediate production environment from which the edible products are derived (soils, irrigation water, aquaculture water and sediments). Surveillance programmes should take into account regional specificities and circumstances when selecting suitable fruit or vegetable products, fish and crustacean species and environmental samples for inclusion in such programmes (Matheu *et al.,* 2017; Dorado-Garcia *et al.,* 2018*).*

Programmes and tools to systematically measure and record antimicrobial contamination and antimicrobial-resistant bacteria in the environment at national levels are virtually absent. Environmental AMR surveillance systems need to be integrated and harmonized with surveillance in the human, animal and food sectors to track the spread of antimicrobial residues, antimicrobial-resistant organisms and ARGs to better assess the risks and priority areas for intervention. A key challenge in this work will be determining an appropriate standard denominator when expressing the magnitude of changes in environmental contamination so that progress within and across countries can be monitored in kind.

4.1 ELEMENTS OF AMR SURVEILLANCE SYSTEM

AMR surveillance on crop, aquaculture and other relevant food production environments would be intended to capture trends over time. It would measure resistance (expressed as percentage resistance of total tested) and enable a comparison among sectors (human, animal, foods of plant origin, foods of aquatic origin). Data captured should also provide information on AMU, so as to enable the identification of public health risks and drivers of resistance. Potential consideration should be given to the practical aspects of data collection. Among these would be the indicator bacteria for each food commodity and environmental sources. Where appropriate, the same bacterial indicator organisms should be used across multiple sample types. For example, although *E. coli* may serve as a suitable common indicator bacterium for antimicrobial-resistant bacteria in foods of animal origin, there is a need to identify additional robust indicators of antimicrobial-resistant bacteria in food of plant origin and in the immediate crop production environment. Likewise, there are no universally accepted bacterial indicators of AMR in aquatic products.

AMR surveillance should use culturing and validated antimicrobial susceptibility testing methods. Standardized panels of antimicrobials have been published (WHO, 2017). Results should subsequently be reported as zone diameters or MICs and interpreted based on epidemiological cut-off values (ECOFFs) (Valsesia *et al.*, 2015). This basic protocol could be expanded to include methods that require more advanced technical complexity and resource requirements – for example, molecular methods for ARG analysis, antimicrobial residue chemical analyses and whole genome sequencing (WGS). AMR profiles are determined for cultured isolates. In addition, metagenomics analyses may provide better insight into the collection of AMR genes that may circulate in the environment, leading to new bacterial serotype- resistance gene combinations, and be transmitted to humans through food consumption – e.g. *E. coli* O104 on sprouts (King *et al.*, 2012; Frank *et al.*, 2011). Based on pilot studies and available data relevant to the commodity and the location, an appropriate sampling plan should be designed for AMR. Chemical residues or the active metabolites themselves should be surveilled, using established standard methods, with the consideration of sampling size and assistance of an epidemiologist and statistician in place for foods of animal origin.

For the foods of plant origin commonly intended to be consumed by animals or by humans, either fresh or minimally processed, consideration should be given to the pathogens of importance to human health and where AMR may be a hazard. These could also include *E. coli* as a measure of hygienic production, *Salmonella* species as an indicator of animal- or human-derived contamination, and *Listeria* species as an environmental indicator of potential contamination with pathogenic *Listeria monocytogenes*, etc. For the foods of plant origin commonly intended to be consumed after cooking, the organisms to be assessed include *E. coli* as a measure of hygienic production, *Salmonella* species as an indicator of human- and animal-derived contamination, and possibly *Pseudomonas* species as a common environmental bacterial contaminant serving as sentinel for AMR.

The surveillance should be tailored to the local agricultural practices since the production systems and food consumption patterns are different in different parts of the world. It should be noted that manure and contaminated water are important vehicles for transferring antimicrobial-resistant bacteria, ARGs and antimicrobial residues from one production system to another, and into the food chain. Knowledge of how animals are managed, housed, fed and how their waste is handled in different regions of the world is critical in designing robust surveillance systems for AMR that will be able to capture points of entry of antimicrobial-resistant bacteria into the food chain. Specimens for consideration to augment and complement activities in terrestrial food animal surveillance include manure solid/liquid used as a fertilizer and samples from human sewage that is used as a fertilizer in some regions. Finally, specific food categories, soils and agricultural water can be included based on their probability of contamination and likelihood of being consumed raw or uncooked (Figure 1, solely as an example).

Antimicrobial-resistant bacteria, ARG and AMU surveillance in fruit and vegetable production systems should capture all important metadata for the antimicrobials such as information from manufacturers, importers and vendors, where possible. Analysis and reporting of these data should be performed on a quarterly basis. If possible, resistance data should also be compared with similar data, coming from human and animal surveillance platforms. Provision of real-time alerts through Rapid Alert System for Food Products (RASFF) might be considered, in the event of a detection of significant threat to public health (e.g. a carbapenemase-producing bacterium) (Florez-Cuadrado *et al.*, 2018).

4.2 ANTIMICROBIAL USE SURVEILLANCE FOR CROP AND AQUACULTURE

Collection of AMU information is an important component of an integrated AMR surveillance programme (WHO, 2017). Representative, population-based

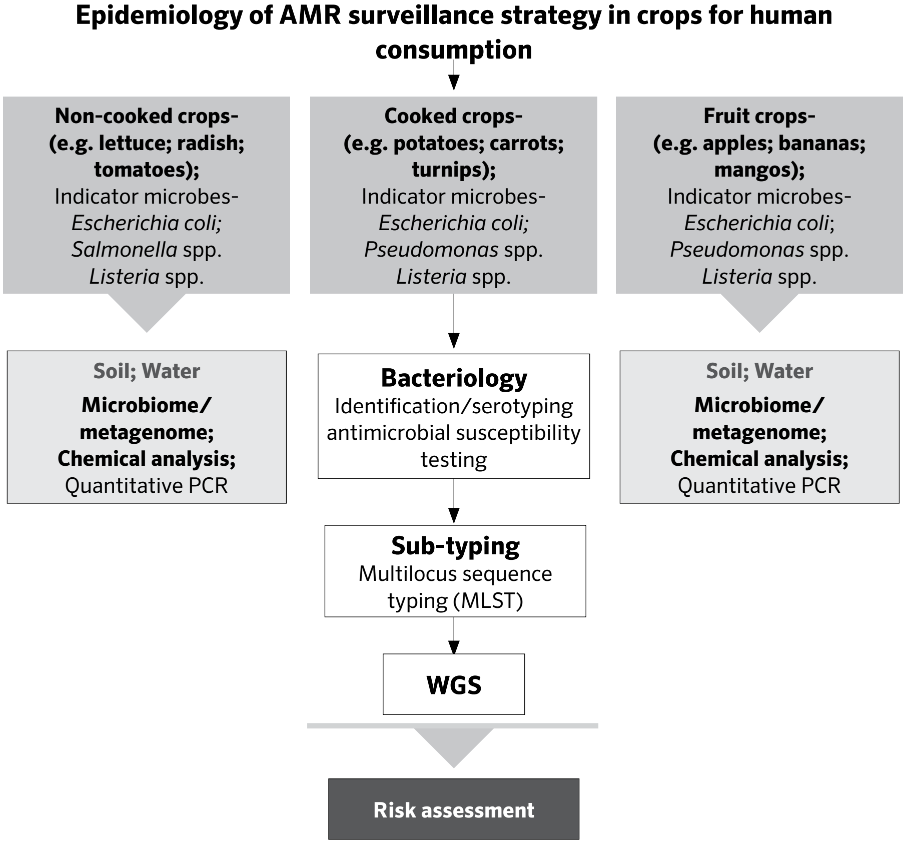


FIGURE 1. Schematic of AMR surveillance strategy in crops for human consumption

AMU data provide information on the patterns and quantities of antimicrobial compounds that are being used in the country or region (European Medicines Agency, 2013). This information is useful for informing AMU policy and for the interpretation of AMR trends. Reducing AMU would be a key indicator to monitor progress of stewardship initiatives at global, country and local levels. Reducing inappropriate use will likely reduce selection pressure and therefore reduce AMR. For example, in some countries AMU surveillance has been used to interpret AMR patterns in foodborne infections of humans (ECDC, EFSA and EMA, 2017) and to support efforts to reduce unnecessary AMU in food-producing animals (SDa Autoriteit Diergeneesmiddelen, 2016). WHO and OIE have published guidelines that apply to the integrated surveillance of AMR, which includes AMU surveillance (WHO, 2017; OIE, 2016). Currently, these guidelines focus on AMU in animal and human sectors, but do not specifically address horticulture, crops and aquaculture.

There is a need to include these sectors in an integrated surveillance model to more comprehensively address AMR from a One Health perspective.

Effective AMU surveillance at the country level should aim to collect data from three sources: from pharmaceutical manufacturers; at the point of sale; and from the end user. This is recommended while recognizing that a wide range of measures – ranging from policy initiatives to farm-level audits – will be required to accomplish this goal. Targeting data from all three levels of the system permits triangulation complementation of information in the event of data gaps.

Manufacturer-level data can be captured through mandating pharmaceutical companies to disclose information annually about the quantity of antimicrobials placed onto the market and for their stated purposes and applications. Furthermore, these data should allow for the disaggregation of information to determine the sector for which the product is purchased (human, animal, crops), the classes of antimicrobials, whether for domestic or export, formations and bulk drugs. These data will extend the understanding of the potential selective pressures being imposed on these ecological niches.

Information acquired at the point of sale should include data on sales from importers, at wholesale outlets and at retail level. Provision should be made at the national and local level to ensure that all necessary documentation related to sales is collected and analyzed to understand sector-specific sales, segregated by type of antimicrobial.

Farm-level usage data and prescription data (in the case of application to aquaculture or crops systems), although potentially difficult to acquire, are necessary to understand actual practices regarding the use of antimicrobials. Provision should be made to ensure farmers and prescribers maintain appropriate records and documentation to describe their antimicrobial compound usage data. These records should be available for audit by competent authorities.

5

Conclusions

5.1 CROPS AS A VEHICLE FOR AMR

- There is clear scientific evidence that foods of plant origin serve as vehicles of foodborne exposure to antimicrobial-resistant bacteria. As such, concerted efforts should be made to mitigate their contamination at all stages of the food chain, from production to consumption.

5.2 FOOD PRODUCTION ENVIRONMENT

- In terrestrial food animal production systems, strict biosecurity should be introduced, including enhanced waste management. Risk assessments should be conducted to identify sources of environmental contamination. AMR surveillance programmes in food animals can be used to inform member countries how to prioritize interventions (production systems and locations) that reduce the need for antimicrobials, thereby reducing the overall burden and transmission of antimicrobial-resistant organisms between animals, crops and the environment.
- Improved methods for infection prevention and control such as husbandry, biosecurity, diagnostics, vaccines, standard methods, testing ideals and other alternatives should be employed to reduce the need for AMU in aquaculture. Consideration should be given to AMR surveillance in aquatic animal food production, in the animals and in the pre- and post-harvest environments. Foods commonly consumed raw – e.g. sushi, oysters, etc. – should be ranked

as highest priority among aquaculture products for inclusion in surveillance programmes. Aquaculture sites should be positioned away from areas of sewage outflow.

- Best management practices should be adhered to with respect to the use of material of human (sewage sludge, biosolids) and animal (manures) origin in primary food production environments. Antimicrobials should only be used in crop production according to label guidelines in the context of IPM strategies.
- Increasing awareness of the issue of antimicrobial residues and antimicrobial-resistant organism contamination in the environment is vital to drive changes in stakeholder practices. Requiring increased transparency on environmental aspects of waste management in food production, processing and pharmaceutical production may further empower consumers to demand products produced by companies that prioritize environmental protection.
- A step-wise approach to antimicrobial stewardship in terrestrial animal food production, crop production and aquaculture provides a strategy for stakeholders to progressively implement changes to control AMR.

5.3 BIOCIDES

- At present, insufficient evidence is available to identify biocide use in food production as a driver of AMR. However, the identified association between biocide tolerance and resistance to one or more classes of antimicrobials underscores the need for increased awareness and prudent use of these products.
- The wide range of biocide applications and targeted bacteria makes it difficult to establish relevant, standardized procedures for biocide susceptibility testing. Nevertheless, harmonized protocols are critically needed for biocide susceptibility testing. Recommendations for methods, ECOFFs, culture methods, biocide storage periods according to the manufacturers' instructions, and control strains, should be developed.
- Monitoring the occurrence of biocide tolerance and cross- and co-resistance in the food production and processing environments should be undertaken. Such monitoring may complement ongoing hygiene and sanitation monitoring programmes for AMR.

5.4 AMR AND AMU SURVEILLANCE

- Plant and aquatic animal food products and their production environments should be integrated into AMU and resistance surveillance programmes to support containment of AMR. The principles and methods used in existing

WHO and OIE guidance should form the basis of AMU surveillance in crops and aquaculture. It is recommended that AMR surveillance be implemented to capture potential seasonal and secular temporal trends. The prevalence of resistant organisms recovered from foods of plant and aquatic animal origin should be measured, using standardized laboratory methods, to enable a direct comparison between domains (human, animal, foods of plant origin and environmental sources) and facilitate identification of public health risks. Isolates of interest should be forwarded to a laboratory with sufficient capacity for confirmation (e.g. National Reference Laboratory) and publicly reported on a quarterly basis.

- Surveillance of AMU and antimicrobial-resistant bacteria in food commodities can provide an assessment of the magnitude of the problem and a tool for measuring progress in mitigation. At a local, regional and global scale there is insufficient knowledge about the amounts and types of antimicrobials applied to crops and those used in terrestrial agriculture and aquaculture. It is recommended that surveillance for AMR and AMU in primary food production environments be implemented in order to obtain additional data that is required for risk assessment and risk management. Terrestrial and aquatic primary food production system environments and products post-harvest should be considered for inclusion in integrated AMU and AMR surveillance programmes that are foundational for containment of AMR.
- The development and enforcement of suitable regulatory instruments may be helpful to address potential misuse of antimicrobials, such as their application to products in the post-harvest period.
- A greater understanding of the role of food production environments in the transmission of foodborne antimicrobial-resistant bacteria and ARGs, and the role of agricultural use of antimicrobials and potential co-selective agents (e.g. copper ions, and potentially other antimicrobials) should incentivize the development of additional tools and strategies to reduce AMU and foodborne AMR.
- Generally, more education and training concerning AMU and AMR should be made available to all stakeholders involved with the use of antimicrobials in production of plant crops and aquaculture (FAO, 2017). To address upstream and downstream contamination of water and soils from human and animal faeces and antimicrobial-resistant organisms and ARGs in the environment, additional training and education on AMR and AMU in terrestrial and aquatic food production systems could also be beneficial.

References

Adams, A. & Thompson, K.D. 2011. Development of diagnostics for aquaculture: challenges and opportunities. *Aquaculture Research*, 42(s1): 93-102.

Agga, G.E., Arthur, T.M., Durso, L.M., Harhay, D.M. & Schmidt, J.W. 2015. Antimicrobial-resistant bacterial populations and antimicrobial resistance genes obtained from environments impacted by livestock and municipal waste. *PLoS One*, 10(7):e0132586.

Aijuka, M., Charimba, G., Hugo, C.J. & Buys, E.M. 2015. Characterization of bacterial pathogens in rural and urban irrigation water. *Journal of Water Health*, 13:103-17.

AMR-Alliance. 2018. AMR industry Alliance antibiotic discharge targets: List of predicted no-effect concentrations (PNECs). https://www.amrindustryalliance.org/wp-content/uploads/2018/09/AMR_Industry_Alliance_List-of-Predicted-No-Effect-Concentrations-PNECs.pdf

Andersson, D.I. & Hughes, D. 2012. Evolution of antibiotic resistance at non-lethal drug concentrations. *Drug Resistance Updates*, 15:162-172. doi:10.1016/j.drup.2012.03.005.

Andersson, D.I. & Hughes, D. 2014. Microbiological effects of sublethal levels of antibiotics. *Nature Reviews Microbiology*, 12:465-478. doi:10.1038/nrmicro3270.

Araujo, S., Silva, I.A.T., Tacao, M., Patinha, C., Alves, A. & Henriques, I. 2017. Characterization of antibiotic resistant and pathogenic *Escherichia coli* in irrigation water and vegetables in household farms. *International Journal of Food Microbiology, 257:192-200.*

Argudín, M.A., Deplano, A., Meghraoui, A., Dodémont, M., Heinrichs, A., Denis, O., Nonhoff, C. & Roisin, S. 2017. Bacteria from animals as a pool of antimicrobial resistance genes. *Antibiotics*, 6(2):12.

Baker-Austin, C., Wright, M.S., Stepanauskas, R. & McArthur, J.V. 2006. Co-selection of antibiotic and metal resistance. *Trends in Microbiology*, 14:176-182. doi:10.1016/j.tim.2006.02.006.

Barancheshme, F. & Munir, M. 2017. Strategies to combat antibiotic resistance in the wastewater treatment plants. *Frontiers in Microbiology, 8:2603.*

Bas, S., Kramer, M. & Stoper, D. 2017. Biofilm surface density determines biocide effectiveness. *Frontiers in Microbiology,* 8:2443.

Bengtsson-Palme, J., Alm Rosenbald, M., Molin, M. & Blomberg, A. 2014. Metagenomics reveals that detoxification systems are underrepresented in marine bacterial communities. *BMC Genomics*, 15:749.

Bengtsson-Palme, J. & Larsson, D.G.J. 2016. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. *Environment International*, 86:140-149. doi:10.1016/j.envint.2015.10.015.

Bennett, P.M. 2008. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology*, 153:S347S357.

Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M.N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F. & Martinez, J.L. 2015. Tackling antibiotic resistance: the environmental framework. *Nature Reviews Microbiology, 13:310-317.*

Berg, J., Thorsen, M.K., Holm, P.E., Jensen, J., Nybroe, O. & Brandt, K.K. 2010. Cu exposure under field conditions coselects for antibiotic resistance as determined by a novel cultivation-independent bacterial community tolerance assay. *Environmental Science and Technology, 4*4:8724-8728.

Bevilacqua, P.D., Bastos, R.K.X. & Mara, D.D. 2014. An evaluation of microbial health risks to livestock fed with wastewater-irrigated forage crops. *Zoonoses and Public Health*, 61:242-249.

Bezanson, G.S., MacInnis, R., Potter, G., & Hughes, T. 2008. Presence and potential for horizontal transfer of antibiotic resistance in oxidase-positive bacteria populating raw salad vegetables. *International Journal of Food Microbiology,* 127:37-42.

Blaak, H., Lynch, G., Italiaander, R., Hamidjaja, R.A., Schets, F.M. & de Roda Husman, A.M. 2015. Multidrug-resistant and extended spectrum beta-lactamase-producing *Escherichia coli* in Dutch Surface water and wastewater. *PLoS One,* 10:e0127752. doi: 10.1371/journal.pone.0127752.

Boehme, S., Werner, G., Klare, I., Reissbrodt, R. & Witte, W. 2004. Occurrence of antibiotic-resistant enterobacteria in agricultural foodstuffs. *Molecular Nutrition and Food Research*, 48:522-531.

Bueno, I., Williams-Nguyen, J., Hwang, H., Sargeant, J.M., Nault, A.J. & Singer, R.S. 2018. Systematic review: Impact of point sources on antibiotic-resistant bacteria in the natural environment. *Zoonoses and Public Health*, 65(1): e162-e184.

Cabello, F.C., Godfrey, H.P., Tomova, A., Ivanova, L., Dölz, H., Millanao, A. & Buschmann, A.H. 2013. Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Environmental Microbiology*, 15(7):1917-1942.

Cabello, F.C., Godfrey, H.P., Buschmann, A.H. & Dölz, H.J. 2016. Aquaculture as yet another environmental gateway to the development and globalisation of antimicrobial resistance. *The Lancet Infectious Diseases*, 16(7):e127-e133.

Chen, C., Ray, P., Knowlton, K. F., Pruden, A. & Xia, K. 2018. Effect of composting and soil type on dissipation of veterinary antibiotics in land-applied manures. *Chemosphere*, 196:270-279.

Chen, J., Wei, X.D., Liu, Y.S., Ying, G.G., Liu, S.S., He, L.Y., Su, H.C., Hu, L.X., Chen, F.R. & Yang, Y.Q. 2016. Removal of antibiotics and antibiotic resistance genes from domestic sewage by constructed wetlands: Optimization of wetland substrates and hydraulic loading. *Science of the Total Environment, 565:240248.*

Chiou, C.S. & Jones, A.L. 1995. Expression and identification of the strA-strB gene pair from streptomycin-resistant *Erwinia amylovora. Gene,* 152(1):47-51.

Christou, A., Agüera, A., Bayona, J.M., Cytryn, E., Fotopoulos, V., Lambropoulou, D., Manaia, C.M., Michael, C., Revitt, M., Schroder, P. & Fatta-Kassinos, D. 2017. The potential implications of reclaimed wastewater reuse for irrigation on the agricultural environment: The knowns and unknowns of the fate of antibiotics and antibiotic resistant bacteria and resistance genes – A review. *Water Research*, 123:448-467.

CLSI. 2015. Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement, Vol. 35. Wayne, PA: Clinical and Laboratory Standards Institute Performance Standards for Document M100S25.

Coleman, B.L., Louie, M., Salvadori, M.I., McEwen, S.A., Neumann, N., Sibley, K., Irwin, R.J., Jamieson, F.B., Daignault, D., Majury, A., Braithwaite, S., Crago, B. & McGeer, A.J. 2013. Contamination of Canadian private drinking water sources with antimicrobial resistant *Escherichia coli. Water Research,* 47:30263036.

Curiao, T., Marchi, E., Grandgirard, D., León-Sampedro, R., Viti, C., Leib, S.L., Baquero, F., Oggioni, M.R., Martinez, J.L. & Coque, T.M. 2016. Multiple adaptive routes of *Salmonella enterica* Typhimurium to biocide and antibiotic exposure. *BMC Genomics*, 17:491. doi: 10.1186/s12864-016-2778-z.

de León, L., Siverio, F., López, M.M. & Rodríguez, A. 2008. Comparative efficiency of chemical compounds for in vitro and in vivo activity against *Clavibacter michiganensis* subsp. *michiganensis*, the causal agent of tomato bacterial canker. *Crop Protection*, 27(9):1277-1283. Doi: 10.1016/j.cropro.2008.04.004

Di Cesare, A., Eckert, E.M., D'Urso, S., Bertoni, R., Gillan, D.C., Wattiez, R. & Corno, G. 2016. Co-occurrence of integrase 1, antibiotic and heavy metal resistance genes in municipal wastewater treatment plants. *Water Research*, 94:208-214. doi:10.1016/j.watres.2016.02.049.

Done, H.Y., Venkatesan, A.K. & Halden, R.U. 2015. Does the recent growth of aquaculture create antibiotic resistance threats different from those associated with land animal production in agriculture? *The AAPS Journal*, 17:513-524

Dorado-García, A., Smid, J.H., van Pelt, W., Bonten, M.J.M., Fluit, A.C., van den Bunt, G., Wagenaar, J.A., Hordijk, J., Dierikx, C.M., Veldman, K.T., de Koeijer, A., Dohmen, W., Schmitt, H., Liakopoulos, A., Pacholewicz, E., Lam, T.J.G.M., Velthuis, A.G., Heuvelink, A., Gonggrijp, M.A., van Duijkeren, E., van Hoek, A.H.A.M., de Roda Husman, A.M., Blaak, H., Havelaar, A.H., Mevius, D.J. & Heederik, D.J.J. 2018. Molecular relatedness of ESBL/AmpC-producing *Escherichia coli* from humans, animals, food and the environment: a pooled analysis. *Journal of Antimicrobial Chemotherapy*. 73(2):339-347.

ECDC (European Centre for Disease Prevention and Control), EFSA (European Food Safety Authority) and EMA (European Medicines Agency). 2017. ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals – Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report. *EFSA Journal* 2017 15:4872, 135 pp.

Elbashir, S., Parveen, S., Schwarz, J., Rippen,T., Jahncke, M. & DePaola, A. 2018. Seafood pathogens and information on antimicrobial resistance: A review. *Food Microbiology*, 70:85-93.

Elsaidy, N., Abouelenien, F. & Kirrella, G.A.K. 2015. Impact of using raw or fermented manure as fish feed on microbial quality of water and fish. *Egyptian Journal of Aquatic Research,* 41(1):93-100.

Evensen, O. 2016. Development of fish vaccines: Focusing on methods in fish vaccines. Chapter 3 in Birkhäuser Advances in Infectious Diseases (A. Adams, ed.) Springer Basel. doi: 10.1007/978-3-0348-0980-1.

European Medicines Agency. 2013. Revised ESVAC reflection paper on collecting data on consumption of antimicrobial agents per animal species, on technical units of measurement and indicators for reporting consumption of antimicrobial agents in animals. doi:EMA/286416/2012-Rev.1.

FAO/WHO. 2003. Code of practice for fish and fishery products. CAC/RCP 52-2003. Rome, FAO.

FAO/OIE/WHO. 2006. Report of a joint FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture and antimicrobial resistance. Seoul, Republic of Korea, 13-16 June 2006.

FAO. 2016a. State of world fisheries and aquaculture 2016. Contributing to food security and nutrition for all. Rome, FAO. 200 pp.

FAO. 2016b. Drivers, dynamics, and epidemiology of AMR in production systems. Rome, FAO.

FAO. 2017. Antimicrobial resistance (AMR) in aquaculture. Rome, FAO. Available at: http://www.fao.org/cofi/46306-0525efdd38c045c798bfbd80a82c2965f.pdf.

FAO. 2018. State of world fisheries and aquaculture 2018. Rome, FAO.

Fenlon, D.R. 1985. Wild birds and silage as reservoirs of Listeria in the agricultural environment. *Journal of Applied Microbiology*, 59(6):537-543.

Fernández-Alarcón, C., Miranda, C.D., Singer, R.S., López, Y., Rojas, R., Bello, H., Domímnguez, M. & González-Rocha, G. 2010. Detection of the floR gene in a diversity of florfenicol resistant Gram-negative bacilli from freshwater salmon farms in Chile. *Zoonoses and Public Health*, 57(3):181-188.

Florez-Cuadrado, D., Moreno, M.A., Ugarte-Ruíz, M. & Domínguez, L. 2018. Antimicrobial resistance in the food chain in the European Union. *Advances in Food and Nutrition Research*, 86:115-136.

Forbes, S., Knight, C.G., Cowley, N.L., Amézquits A., McClure, P., Hunphreys, G. & McBain, A.J. 2016. Variable effects of exposure to formulated microbicides on antibiotic susceptibility in firmicutes and proteobacteria. *Applied and Environmental Microbiology*, 82(12):3591-3598.

Frank, C., Werber, D., Cramer, J. P., Askar, M., Faber, M., an der Heiden, M., Bernard, H., Fruth, A., Prager, R., Spode, A., Wadl, M., Zoufaly, A., Jordan, S., Kemper, M.J., Follin, P., Muller, L., King, L.A., Rosner, B., Buchholz, U., Stark, K. & Krause, G. 2011. Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. *The New England Journal of Medicine*, 365:1771-80.

Gullberg, E., Albrecht, L.M., Karlsson, C., Sandegren, L. & Andersson, D.I. 2014. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. *mBio*, 5:e01918–14. doi:10.1128/mBio.01918-14.

Gullberg, E., Cao, S., Berg, O.G., Ilbäck, C., Sandegren, L., Hughes, D. & Andersson, D.I. 2011. Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathogens*, 7:e1002158. doi:10.1371/journal.ppat.1002158.

Hardy, K., Sunnucks, K., Gil, H., Shabir, S., Trampari, E., Hawkey, R. & Webber, M. 2018. Increased usage of antiseptics is associated with reduced susceptibility in clinical isolates of *Staphylococcus aureus. mBio,* 9(3):e00894-18.

Hasman, H., Kempf, I., Chidaine, B., Cariolet, R., Ersbøll, A.K., Houe, H., Bruun Hansen, H.C. & Aarestrup, F.M. 2006. Copper resistance in *Enterococcus faecium*, mediated by the tcrB gene, is selected by supplementation of pig feed with copper sulfate. *Applied and Environmental Microbiology*, 2006. 72(9):5784-5789.

Hassan, S.A., Altalhi, A.D., Gherbawy, Y.A. & El-Deeb, B.A. 2011. Bacterial load of fresh vegetables and their resistance to the currently used antibiotics in Saudi Arabia. *Foodborne Pathogens and Disease*, 8:1011-1018.

He, X., Xu, Y., Chen, J., Ling, J., Li, Y., Huang, L., Zhou, X., Zheng, L. & Xie, G. 2017. Evolution of corresponding resistance genes in the water of fish tanks with multiple stresses of antibiotics and heavy metals. *Water Research*, 124:39-48.

Heyndrickx, M. 2011. The importance of endospore-forming bacteria originating from soil for contamination of industrial food processing. *Applied and Environmental Soil Science*, 561975. Available at: https://doi.org/10.1155/2011/561975.

Hughes, D. & Andersson, D.I. 2012. Selection of resistance at lethal and non-lethal antibiotic concentrations. *Current Opinion in Microbiology*, 15:555-560. doi:10.1016/j.mib.2012.07.005.

Jang, H.M., Kim, Y.B., Choi, S., Lee, Y., Shin, S.G., Unno, T. & Kim, Y.M. 2018. Prevalence of antibiotic resistance genes from effluent of coastal aquaculture, South Korea. *Environmental Pollution,* 233:10491057.

Jechalke, S., Kopmann, C., Rosendahl, I., Groeneweg, J., Weichelt, V., Krögerrecklenfort, E., Brandes, N., Nordwig, M., Ding, G.C., Siemens, J., Heuer, H. & Smalla, K. 2013. Increased abundance and transferability of resistance genes after field application of manure from sulfadiazine-treated pigs. *Applied and Environmental Microbiology*, 79:1704-1711.

Jongman, M. & Korsten, L. 2016. Genetic diversity and antibiotic resistance of *Escherichia coli* isolates from different leafy green production systems. *Journal of Food Protection*, 79:1846-1853.

Joy, S.R., Bartelt-Hunt, S.L., Snow, D.D., Gilley, J.E., Woodbury, B.L., Parker, D., David B. Marx, D.B. & Li, X. 2013. Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry. *Environmental Science and Technology*, 47: 12081-12088.

Karkman, A., Do, T.T., Walsh, F. & Virta, M.P.J. 2018. Antibiotic-resistance genes in waste water. *Trends in Microbiology,* 26:220-228.

Khan, S., Beattie, T.K. & Knapp, C.W. 2017. The use of minimum selectable concentrations (MSCs) for determining the selection of antimicrobial resistant bacteria. *Ecotoxicology*, 26:283-292. doi:10.1007/s10646-017-1762-y.

King, L.A., Nogareda, F., Weill, F. X., Mariani-Kurkdjian, P., Loukiadis, E., Gault, G., Jourdan-Dasilva, N., Bingen, E., Mace, M., Thevenot, D., Ong, N., Castor, C., Noel, H., Van Cauteren, D., Charron, M., Vaillant, V., Aldabe, B., Goulet, V., Delmas, G., Couturier, E., Le Strat, Y., Combe, C., Delmas, Y., Terrier, F., Vendrely, B., Rolland, P. & De Valk, H. 2012. Outbreak of Shiga toxin-producing *Escherichia coli* O104:H4 associated with organic fenugreek sprouts, France, June 2011. *Clinical Infectious Diseases*, 54:1588-94.

Kraupner, N., Ebmeyer, S., Bengtsson-Palme, J., Fick, J., Kristiansson, E., Flach, C.F. & Larsson, D.G.J. 2018. Selective concentration for ciprofloxacin resistance in *Escherichia coli* grown in complex aquatic bacterial biofilms. *Environment International*, 116:255-268. doi:10.1016/j.envint.2018.04.029.

Krzeminski, P., Tomei, M.C., Karaolia, P., Langenhoff, A., Almeida, C.M.R., Felis, E., Gritten, F., Andersen, H.R., Fernandes, T., Manaia, C.M., Rizzo, L. & Fatta-Kassinos, D. 2019. *Science of the Total Environment*, 648:1052-1081.

Lamichhane, J.R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J.B. & Aubertot, J.-N. 2018. Thirteen decades of antimicrobial copper compounds applied in agriculture: A review. *Agronomy for Sustainable Development, 38:28.*

LaPara, T.M., Burch, T.R., McNamara, P.J., Tan, D.T., Yan, M. & Eichmiller, J.J. 2011. Tertiary-treated municipal wastewater is a significant point-source of antibiotic resistance genes into Duluth-Superior Harbor. *Environmental Science and Technology,* 45:9543-9549.

Liu, A., Fong, A., Becket, E., Yuan, J., Tamae, C., Medrano, L., Maiz, M., Wahba, C., Lee, C., Lee, K., Tran, K.P., Yang, H., Hoffman, R.M., Salih, A. & Miller, J.H. 2011. Selective advantage of resistant strains at trace levels of antibiotics: a simple and ultrasensitive color test for detection of antibiotics and genotoxic agents. *Antimicrobial Agents and Chemotherapy*, 55:1204-1210. doi:10.1128/AAC.01182-10.

Liu, J., Zhao, Z., Orfe, L., Subbiah, M. & Call, D.R. 2016. Soil-borne reservoirs of antibiotic-resistant bacteria are established following therapeutic treatment of dairy calves. *Environmental Microbiology*, 18:557-564.

Marti, R., Scott, A., Tien, Y.-C., Murray, R., Sabourin, L., Zhang, Y. & Topp, E. 2013. Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. *Applied and Environmental Microbiology*, 79:57015709.

Marti, R., Tien, Y.-C., Murray, R., Scott, A., Sabourin, L. & Topp, E. 2014. Safely coupling livestock and crop production systems: how rapidly do antibiotic resistance genes dissipate in soil following a commercial application of swine or dairy manure? *Applied and Environmental Microbiology*, 80:32583265.

Marshall, B.M. & Levy, S.B. 2011. Food animals and antimicrobials: impacts on human health. *Clinical Microbiology Reviews*, 24:718-733. doi:10.1128/cmr.00002- 11.

Matheu, J., Aidara-Kane, A. & Andremont, A. 2017. The ESBL tricycle amr surveillance project: a simple, one health approach to global surveillance. *AMR Control*, 2017. Available at: http://resistancecontrol.info/2017/the-esbl-tricycle-amr-surveillance-project-a-simple-one-health-approach-to-global-surveillance/.

McManus, P.S., Stockwell, V.O., Sundin, G.W. & Jones, A.L. 2002. Antibiotic use in plant agriculture. *Annual Review of Phytopathology*, 40:443-465.

Mezger, A., Gullberg, E., Göransson, J., Zorzet, A., Herthnek, D., Tano, E., Nilsson, M. & Andersson, D.I. 2015. A general method for rapid determination of antibiotic susceptibility and species in bacterial infections. *Journal of Clinical Microbiology*, 53:425-432. doi:10.1128/JCM.02434-14.

Minich, J.J., Zhu, Q., Xu, Z.Z., Amir, A., Ngochera, M., Simwaka, M., Allen, E.E., Zidana, H. & Knight, R. 2018. Microbial effects of livestock manure fertilization on freshwater aquaculture ponds rearing tilapia (*Oreochromis shiranus*) and North African catfish (*Clarias gariepinus*). *MicrobiologyOpen.* e716. Available at: https://doi.org/10.1002/mbo3.716.

Miranda, C. D. & Zemelman, R. 2002. Bacterial resistance to oxytetracycline in Chilean salmon farming. *Aquaculture*, 212(1-4):31-47.

Mourão, J., Novais, C., Machado, J., Peixe, L. & Antunes, P. 2015. Metal tolerance in emerging clinically relevant multidrug-resistant *Salmonella enterica* serotype 4,[5],12:i:– clones circulating in Europe. *International Journal of Antimicrobial Agents*, 45(6):610-616.

MPI [Ministry for Primary Industries]. 2016. 2011-2014 antibiotic sales analysis. Available at: https://www.mpi.govt.nz/dmsdocument/14497/loggedIn.

Murray, A., Zhang, L., Yin, X., Zhang, T., Buckling, A., Snape, J. & Gaze, W.H. 2018. Novel insights into selection for antibiotic resistance in complex microbial communities. *mBio*, 9(4):E00969-18. doi:10.1101/323634.

Muurinen, J., Stedtfeld, R., Karkman, A., Pärnänen, K., Tiedje, J. & Virta, M. 2017. Influence of manure application on the environmental resistome under Finnish agricultural practice with restricted antibiotic use. *Environmental Science and Technology*, 51(11):5989-5999.

Ngo, T.P., Smith, P., Bartie, K.L., Thompson, K.D., Verner-Jeffreys, D.W., Hoare, R. & Adams, A. 2018. Antimicrobial susceptibility of *Flavobacterium psychrophilum* isolates from the United Kingdom. *Journal of Fish Diseases*, 41(2):309-320.

Nightingale, K.K., Schukken, Y.H., Nightingale, C.R., Fortes, E.D., Ho, A.J., Her, Z., Grohn, Y.T., McDonough, P.L. & Wiedmann, M. 2004. Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Applied and Environmental Microbiology*, 70(8):4458-4467.

Njage, P.M.K. & Buys, E.M. 2015. Pathogenic and commensal *Escherichia coli* from irrigation water show potential in transmission of extended spectrum and AmpC β-lactamases determinants to isolates from lettuce. *Microbial Biotechnology*, 8:462-473.

Norelli, J.L., Burr, T.J., Lo Cicero, A.M., Gilbert, M.T. & Katz, B.H. 1991. Homologous streptomycin resistance gene present among diverse Gram-negative bacteria in New York State apple orchards. *Applied and Environmental Microbiology*, 57(2):486-491.

Norwegian Veterinary Institute. 2017. Antimicrobial resistance in the Norwegian environment – red fox as an indicator.Report 11-2017.

Novais, C., Campos, J., Freitas, A., Barros, M., Sileira, E., Coque, T., Antunes, P. & Peixe. 2018. Water supply and feed as sources of antimicrobial-resistant *Enterococcus* spp. in aquacultures of rainbow trout (*Oncorhyncus mykiss*), Portugal. *Science of the Total Environment*, 625:1102-1112.

OIE [World Organisation for Animal Health]. 2016. OIE annual report on the use of antimicrobial agents in animals. Paris.

Oxaran, V., Dittmann, K.K., Lee, S.H., Chaul, L.T., de Oliveira, C.A.F., Corassin, C.H., Alves V.F., De Martinis, E.C.P. & Gram, L. 2018. Behavior of foodborne pathogens, *Listeria monocytogenes* and *Staphylococcus aureus,* in mixed-species biofilm exposed to biocides. *Applied and Environmental Microbiology*, AEM. 02038-18.

Pan, M. & Chu, L.M. 2018. Occurrence of antibiotics and antibiotic resistance genes in soils from wastewater irrigation areas in the Pearl River Delta region, southern China. *Science of the Total Environment*, 624:145-152.

Pal, C., Bengtsson-Palme, J., Kristiansson, E. & Larsson, D.G.J. 2015. Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. *BMC Genomics*, 16:964. doi:10.1186/s12864-015-2153-5.

Palacios, O.A., Contreras, C.A., Muñoz-Castellanos, L.N., González-Rangel, M.O., Rubio-Arias, H., Palacios-Espinosa, A. & Nevárez-Moorillón, G.V. 2017. Monitoring of indicator and multidrug resistant bacteria in agricultural soils under different irrigation patterns. *Agricultural Water Management*, 184:1927.

Peters, K., Pazos, M., Edoo, Z., Hugonnet, J.E., Martorana, A.M., Polissi, A., VanNieuwenhze, M.S., Arthur, M. & Vollmer, W. 2018. Copper inhibits peptidoglycan LD-transpeptidases suppressing β-lactam resistance due to bypass of penicillin-binding proteins. *Proceedings of the National Academy of Sciences*, 115(42):10786-10791.

Petersen, A., Andersen, J. S., Kaewmak, T., Somsiri, T. & Dalsgaard, A. 2002. Impact of integrated fish farming on antimicrobial resistance in a pond environment. *Applied and Environmental Microbiology*, 68(12):6036-6042.

Pool, K. 2017. At the nexus of antibiotics and metals: the impact of Cu and Zn on antibiotic activity and resistance. *Trends Microbiol,* 25:820-832. doi: 10.1016/j.tim.2017.04.010.

Pope, L., Boxall, A.B.A., Corsing, C., Halling-Sorensen, B., Tait, A. & Topp, E. 2009. Exposure assessment of veterinary medicines in terrestrial systems. *In:* Veterinary Medicines in the Environment. (M. Crane, A.B.A. Boxall & K. Barrett, eds.) SETAC, Pensacola, FL, USA 2009, pp. 129-154.

Pourcher, A.M., Jadas-Hécart, A., Cotinet, P., Dabert, P., Ziebal, C., Le Roux, S., Moraru, R., Heddadj, D. & Kempf, I. 2014. Effect of land application of manure from enrofloxacin-treated chickens on ciprofloxacin resistance of Enterobacteriaceae in soil. *Science of the Total Environment*, 482-483:269-275.

Pruden, A., Pei, R.T., Storteboom, H., & Carlson, K.H. 2006. Antibiotic resistance genes as emerging contaminants: Studies in northern Colorado. *Environmental Science and Technology*, 40: 7445-7450.

Rahube, T.O., Marti, R., Scott, A., Tien, Y.-C., Murray, R., Sabourin, L., Zhang, Y., Duenk, P., Lapen, D.R. & Topp, E. 2014. Impact of fertilizing with raw or anaerobically digested sewage sludge on the abundance of antibiotic-resistant coliforms, antibiotic resistance genes, and pathogenic bacteria in soil and on vegetables at harvest. *Applied and Environmental Microbiology*, 80:6898-6907.

Raphael, E., Wong, L.K. & Riley, L.W. 2011. Extended-spectrum Beta-lactamase gene sequences in gram-negative saprophytes on retail organic and nonorganic spinach. *Applied and Environmental Microbiology*, 77:1601-1607.

Rodríguez, C., Lang, L., Wang, A., Altendorf, K., García, F. & Lipski, A. 2006. Lettuce for human consumption collected in Costa Rica contains complex communities of culturable oxytetracycline- and gentamicin-resistant bacteria. *Applied and Environmental Microbiology*, 72:5870-5876.

Romero, J.L., Grande Burgos, M.J., Pérez-Pulido, R., Gálvez, A. & Lucas, R. 2017. Resistance to antibiotics, biocides, preservatives and metals in bacteria isolated from seafoods: co-selection of strains resistant or tolerant to different classes of compounds. *Frontiers in Microbiology*, 8:1650.

Ruimy, R., Brisabois, A., Bernede, C., Skurnik, D., Barnat, S., Arlet, G., Momcilovic, S., Elbaz, S., Moury, F., Vibet, M.A., Courvalin, P., Guillemot, D. & Andremont, A. 2010. Organic and conventional fruits and vegetables contain equivalent counts of gram-negative bacteria expressing resistance to antibacterial agents. *Environmental Microbiology*, 12:608-615.

Sandegren, L. 2014. Selection of antibiotic resistance at very low antibiotic concentrations. *Upsala Journal of Medical Sciences*, 119:103-107. Available at: doi:10.3109/03009734.2014.904457.

Schnabel, E.L., & Jones, A.L. 1999. Distribution of tetracycline resistance genes and transposons among phylloplane bacteria in Michigan apple orchards. *Applied and Environmental Microbiology,* 65:48984907.

Schwaiger, K., Helmke, K., Hölzel, C.S. & Bauer, J. 2011. Antibiotic resistance in bacteria isolated from vegetables with regards to the marketing stage (farm vs. supermarket). *International Journal of Food Microbiology*, 148:191-196.

SDa Autoriteit Diergeneesmiddelen. 2016. Usage of antibiotics in agricultural livestock in the Netherlands in 2015: Trends, benchmarking of livestock farms and veterinarians, and a revision of the benchmarking method. The Netherlands Veterinary Medicines Authority, Utrecht. Available at: http://www.autoriteitdiergeneesmiddelen.nl/Userfiles/Eng%20rapport%20AB%202016/engels-def-rapportage-2016-deel-1-en-2-22-09-2017.pdf.

Seiler, C. & Berendonk, T.U. 2012. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Frontiers in Microbiology*, 3:399.

Silveira, E., Freitas, A.R., Antunes, P., Barros, M., Campos, J., Coque, T.M., Peixe, L. & Novais, C. 2014. Co-transfer of resistance to high concentrations of copper and first-line antibiotics among Enterococcus from different origins (humans, animals, the environment and foods) and clonal lineages. *Journal of Antimicrobial Chemotherapy*, 69:899-906.

Singer, A.C., Shaw, H., Rhodes, V. & Hart, A. 2016. Review of antimicrobial resistance in the environment and its relevance to environmental regulators. *Frontiers in Microbiology*, 7:1728.

Singh, S., Lee, M.H., Park, I., Shin, Y. & Lee, Y.S. 2016. Antimicrobial seafood packaging: a review. *Journal of Food Science and Technology*, 53(6):2505-2518.

Sobiczewski, P., Chiou, C.S. & Jones, A.L. 1991. Streptomycin-resistant epiphytic bacteria with homologous DNA for streptomycin resistance in Michigan apple orchards. *Plant Disease*, 75(11):11101113.

Stärk, K. D., Pękala, A. & Muellner, P. 2019. Use of molecular and genomic data for disease surveillance in aquaculture: Towards improved evidence for decision making. *Preventive Veterinary Medicine,* in press. https://doi.org/10.1016/j.prevetmed.2018.04.011.

Stockwell, V.O. & Duffy, B. 2012. Use of antibiotics in plant agriculture. *Scientific and Technical Review*, 31(1):199-210.

Strukova, E.N., Portnoy, Y.A., Zinner, S.H. & Firsov, A.A. 2016. Predictors of bacterial resistance using in vitro dynamic models: area under the concentration-time curve related to either the minimum inhibitory or mutant prevention antibiotic concentration. *Journal of Antimicrobial Chemotherapy*, 71:678-684. doi:10.1093/jac/dkv387.

Sundin, G.W. & Bender, C. 1993. Ecological and genetic analysis of copper and streptomycin resistance in *Pseudomonas syringae pv. syringae. Applied and Environmental. Microbiology,* 59:1018-1024.

Sundin, G.W., Monks, D. & Bender, C. 1995. Distribution of the streptomycin-resistance transposon TN5393 among phylloplane and soil bacteria from managed agricultural habitats. *Canadian Journal of Microbiology,* 41:792-799.

Sundin, G.W. & Wang, N. 2018. Antibiotic resistance in plant-pathogenic bacteria. *Annual Review of Phytopathology*, 56:161-180. doi: 10.1146/annurev-phyto-080417-045946.

Surette, M.D. & Wright, G.D. 2017. Lessons from the environmental antibiotic resistome. *Annual Review of Microbiology,* 71:309-329. doi:10.1146/annurev-micro-090816-093420.

Tendencia, E.A. & de la Peña, L.D. 2001. Antibiotic resistance of bacteria from shrimp ponds. *Aquaculture*, 195(3-4):193-204.

Thanner, S., Drissner, D. & Walsh F. 2016. Antimicrobial resistance in agriculture. *mBio*, 7(2):e02227-15.

Valsesia, G., Hombach, M., Maurer, F.P., Courvalin, P., Roos, M. & Böttger, E.C. 2015. The resistant-population cutoff (RCOFF): a new concept for improved characterization of antimicrobial susceptibility patterns of non-wild-type bacterial populations. *Journal of Clinical Microbiology*, 53(6):1806-11.

Verraes, C., Boxstael, S.V., Meervenne, E.V., Coillie,E.V., Butaye, P., Catry,B., Schaetzen,M.-A., Huffel, X.V., Imberechts, H., Dierick, K., Daube, G., Saegerman, C., Block, J.D., Dewulf, J. & Herman, L. 2013. Antimicrobial resistance in the food chain: A review. *International Journal of Environmental Research and Public Health*, 10(7):2643-2669.

Versluis, D., D'Andrea, M.M., Garcia, J.R., Leimena, M.M., Hugenholtz, F., Zhang, J., Öztürk, B., Nylund, L., Sipkema, D., van Schaik, W., de Vos, W.M., Kleerebezem, M., Smidt, H. & van Passel, M.W.J. 2015. Mining microbial metatranscriptomes for expression of antibiotic resistance genes under natural conditions. *Scientific Reports*, 5:11981.

Wales, A.D. & Davies, R.H. 2015. Co-selection of resistance to antibiotics, biocides and heavy metals, and its relevance to foodborne pathogens. *Antibiotics, 4(4):567-604.*

Walia, S., Rana, S.W., Maue, D., Rana, J., Kumar, A. & Walia, S.K. 2013. Prevalence of multiple antibiotic-resistant Gram-negative bacteria on bagged, ready-to-eat baby spinach. *International Journal of Environmental Health Research*, 23:108-118.

Watts, J.E., Schreier, H.J., Lanska, L. & Hale, M. S. 2017. The rising tide of antimicrobial resistance in aquaculture: sources, sinks and solutions. *Marine Drugs*, 15(6):158.

Webber, M.A., Whitehead, R.N., Mount, M., Loman, N.J., Pallen, M.J. & Piddock, L.J. 2015. Parallel evolutionary pathways to antibiotic resistance selected by biocide exposure. Journal of Antimicrobial Chemotherapy, 70(8):2241-2248.

Wellington, E.M., Boxall, A.B., Cross, P., Feil, E.J., Gaze, W.H., Hawkey, P.M., Johnson-Rollings, A.S., Jones, D.L., Lee, N.M., Otten, W., Thomas, C.M. & Williams, A.P. 2013. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. T*he Lancet Infectious Diseases*, 13:155-165.

WHO. 2017. Integrated surveillance of antimicrobial resistance in foodborne bacteria: Application of a One Health approach. Guidance from a WHO Advisory Group. World Health Organization, Advisory Group on Integrated Surveillance of Antimicrobial Resistance, Geneva.

Witte, W. 2000. Ecological impact of antibiotic use in animals on different complex microflora: environment. *International Journal of Antimicrobial Agents*, 14:321-325. doi:10.1016/S0924- 8579(00)00144-8.

Wright, G.D. 2010. Antibiotic resistance in the environment: a link to the clinic? *Current Opinion in Microbiology*, 13:589-594. doi:10.1016/j.mib.2010.08.005.

Xie, W.Y., Shen, Q. & Zhao, F.J. 2018. Antibiotics and antibiotic resistance from animal manures to soil: a review. *European Journal of Soil Science*, 69(1):181-195.

Yu, Z., Gunn, L. & Fanning, S. 2017. Antimicrobial resistance and its association with tolerance to heavy metals in agriculture production. *Food Microbiology*, 64:23-32.

Zarfel, G., Krziwanek, K., Johler, S., Hoenigl, M., Leitner, E., Kittinger, C., Masoud, L., Feierl, G. & Grisold, A.J. 2013. Virulence and antimicrobial resistance genes in human MRSA ST398 isolates in Austria. *Epidemiology and Infection*, 141(4):888-892.

Zhao, Z., Wang, J., Han, Y., Chen, J., Liu, G., Lu, H., Yan, B. & Chen, S. 2017. Nutrients, heavy metals and microbial communities co-driven distribution of antibiotic resistance genes in adjacent environment of mariculture. *Environmental Pollution*, 220:909-918. doi:10.1016/j.envpol.2016.10.075.

Zhou, X., Qiao, M., Wang, F.-H. & Zhu, Y.-G. 2017a. Use of commercial organic fertilizer increases the abundance of antibiotic resistance genes and antibiotics in soil. *Environmental Science and Pollution Research,* 24:701-710.

Zhou, Y., Niu, L., Zhu, S., Lu, H. & Liu, W. 2017b. Occurrence, abundance, and distribution of sulfonamide and tetracycline resistance genes in agricultural soils across China. *Science of the Total Environment*, 599-600:1977-1983.

Zhu, Y.-G., Johnson, T.A., Su, J.-Q., Qiao, M., Guo, G.-X., Stedtfeld, R.D., Hashsham, S.A. & Tiedje, J.M. 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proceedings of the National Academy of Sciences*, 110:3435-3440.

FAO/WHO Microbiological Risk Assessment Series

1 Risk assessments of *Salmonella* in eggs and broiler chickens: Interpretative Summary, 2002

2 Risk assessments of *Salmonella* in eggs and broiler chickens, 2002

3 Hazard characterization for pathogens in food and water: Guidelines, 2003

4 Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: Interpretative Summary, 2004

5 Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: Technical Report, 2004

6 *Enterobacter sakazakii* and microorganisms in powdered infant formula: Meeting Report, 2004

7 Exposure assessment of microbiological hazards in food: Guidelines, 2008

8 Risk assessment of *Vibrio vulnificus* in raw oysters: Interpretative Summary and Technical Report, 2005

9 Risk assessment of choleragenic *Vibrio cholerae* 01 and 0139 in warm-water shrimp in international trade: Interpretative Summary and Technical Report, 2005

10 *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: Meeting Report, 2006

11 Risk assessment of *Campylobacter* spp. in broiler chickens: Interpretative Summary, 2008

12 Risk assessment of *Campylobacter* spp. in broiler chickens: Technical Report, 2008

13 Viruses in food: Scientific Advice to Support Risk Management Activities: Meeting Report, 2008

14 Microbiological hazards in fresh leafy vegetables and herbs: Meeting Report, 2008

15 *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered follow-up formula: Meeting Report, 2008

16 Risk assessment of *Vibrio parahaemolyticus* in seafood: Interpretative Summary and Technical Report, 2011

17 Risk characterization of microbiological hazards in food: Guidelines, 2009.

18 Enterohaemorragic *Escherichia coli* in meat and meat products: Meeting Report, 2010

19 *Salmonella* and *Campylobacter* in chicken meat: Meeting Report, 2009

20 Risk assessment tools for *Vibrio parahaemolyticus* and *Vibrio vulnificus* associated with seafood: Meeting Report and Follow-up, In press

21 *Salmonella* spp. In bivalve molluscs: Risk Assessment and Meeting Report, In press

22 Selection and application of methods for the detection and enumeration of human pathogenic *Vibrio* spp. in seafood: Guidance, 2016

23 Multicriteria-based ranking for risk management of food-borne parasites, 2014

24 Statistical aspects of microbiolgical criteria related to foods: A risk managers guide, 2016

25 A risk based approach for the control of *Trichinella* in pigs and *Taenia saginata* in beef: Meeting Report, In press

26 Ranking of low moisture foods in support of microbiological risk management: Meeting Report and Systematic Review, In press

27 Microbiological hazards associated with spices and dried aromatic herbs: Meeting Report, In press

28 Microbial Safety of lipid based ready-to-use foods for the management of moderate acute and severe acute malnutrition: First meeting report, 2016

29 Microbial Safety of lipid based ready-to-use foods for the management of moderate acute and severe acute malnutrition: Second meeting report, In press

30 Interventions for the Control of Non-typhoidal *Salmonella* spp. in Beef and Pork: Meeting Report and Systematic Review, 2016

31 Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring, 2018

32 Attributing illness caused by Shiga toxin-producing *Escherichia coli* (STEC) to specific foods : Report, 2019

33 Safety and Quality of Water Used in Food Production and Processing : Meeting Report, 2019

34 Joint FAO/WHO Expert Meeting in collaboration with OIE on Foodborne Antimicrobial Resistance: Role of the Environment, Crops and Biocides : Meeting Report, 2019

Microbiological Risk
Assessment Series

In recognition of the growing problem of antimicrobial resistance (AMR), its increasing threat to human, animal and plant health, and the need for a One Health approach to address this issue, the 39th Session of the Codex Alimentarius Commission (CAC) highlighted the importance for the food safety community to play its part and agreed to re-establish the ad hoc Codex Intergovernmental Task Force on Antimicrobial Resistance (TFAMR). The objectives of the TFAMR were to revise the Codex Code of Practice to Minimise and Contain Antimicrobial Resistance (CAC/RCP 61-2005) and to develop a Codex guidance on integrated surveillance of AMR. Responding to the request from the CAC and the TFAMR to provide scientific advice in the areas of crops, environment and biocides, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) convened, in collaboration with the World Organisation for Animal Health (OIE), a joint "FAO/WHO expert meeting on foodborne antimicrobial resistance: role of environment, crops and biocides" on 11-15 June 2018 in Rome, Italy.

This report summarises the outcome of that meeting.

For further information on the joint FAO/WHO activities on microbiological risk assessment and related areas, please contact

Office of Food Safety
Agriculture and Consumer Protection Department
Food and Agriculture Organization of the United Nations
Viale delle Terme di Caracalla
00153 Rome, Italy
Email: jemra@fao.org
Website: www.fao.org/food/food-safety-quality

Department of Food Safety and Zoonoses
World Health Organization
20 Avenue Appia
1211 Geneva 27, Switzerland
Email: foodsafety@who.int
Website: www.who.int/foodsafety

ISBN 978-92-5-131890-4 ISSN 1726-5274
9 789251 318904
CA6724EN/1/10.19