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Global Perspectives on Antimicrobial Resistance in the Food Chain

ABSTRACT

Antimicrobial resistance (AMR) has become a priority issue in human medicine and agri-food systems, with many countries recognizing it as an important emerging threat to global public health and food security. In the past few years, a concerted global effort has attempted to bring this issue into the limelight and secure the political commitment to take action. While development of resistance is a natural phenomenon, overuse and inappropriate use of antimicrobials are important factors in rapidly exacerbating the problem. Antimicrobials, an important part of our food and agriculture production systems, are used for both therapeutic and non-therapeutic purposes. With the increasing demand for food, particularly of animal origin, to meet the demands of a growing global population, antimicrobial use in the food and agriculture sector is expected to rise substantially in some parts of the world. Meeting the dual challenge of protecting the efficacy of antimicrobials while still producing adequate safe food is a major task facing the food and agriculture sector. This paper explores some of the issues around AMR in the food and agriculture sectors, with a particular focus on AMR in aspects of food and food safety along the food chain.

INTRODUCTION

The availability and use of antimicrobial drugs in terrestrial and aquatic animals and crop production is essential for the treatment of plant and animal infections and thereby contributes to animal health and welfare and plant health. This, in turn, contributes to the sustainability of production, the protection of livelihoods of those involved in food production, and ultimately food security *(13)*. There are global concerns that growing resistance among bacteria to antimicrobial drugs in these food and agriculture sectors threatens to reverse these benefits as well as the benefits to human health that have been seen for decades. Antimicrobial resistance (AMR) has thus become a priority issue in human medicine, animal health and the wider food and agriculture sector *(12, 39)*.

In May 2014, following the publication of the first global report on surveillance on AMR *(40)*, the World

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Health Assembly adopted a Resolution that called for the development of a Global Action Plan (GAP) on AMR and strengthening of collaboration among what are often referred to as the tripartite: the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), and the World Organization for Animal Health (OIE) to combat AMR within the context of the "One Health" approach. The GAP, which was developed by WHO with inputs from FAO and OIE and which was adopted in 2015 *(41)*, sets out five strategic objectives: (1) to improve awareness and understanding of AMR; (2) to strengthen knowledge through surveillance and research; (3) to reduce the incidence of infection; (4) to optimize the use of antimicrobial agents, and (5) to develop the economic case for sustainable investment that takes account of the needs of all countries and increases investment in new medicines, diagnostic tools, vaccines and other interventions. It clearly recognizes a role for the food and agriculture sectors in multi-sectoral global efforts to combat AMR. Further, in September, 2016, world leaders at the United Nations General Assembly recognized the importance of AMR as a health issue and committed to *"taking a broad, coordinated approach to address the root causes of AMR across multiple sectors, especially human health, animal health, and agriculture" (42)*.

GLOBAL COMMITMENT TO AMR IN THE FOOD AND AGRICULTURE SECTOR

Highlighting the importance of this issue for the food and agriculture sector, FAO members in 2015 adopted a Resolution on AMR *(12)* that set out targets for the membership and the FAO. The commitment of OIE was also concretized through the adoption of a resolution on AMR in 2015 *(30)*. The challenge now is to channel and convert the high-level political commitment to this issue into real action. To address this, FAO has defined and published its strategy through its plan of action to support the food and agriculture sector in addressing AMR. FAO's plan revolves around four pillars: (1) awareness of the issue, its impact on the sector and commitment to action; (2) evidence (through surveillance and monitoring) to support the development of locally relevant strategies and data against which to measure progress; (3) governance both locally and internationally, and (4) the promotion of good practices in food and agricultural systems to reduce the use of antimicrobials and the spread of antimicrobial resistance *(Fig. 1) (14)*. OIE has also recently published its strategy on antimicrobial resistance *(31)*.

The complexity and interconnectivity of the environment in which AMR develops and is disseminated means that the success of any effort depends on achieving effective

Figure 1. The four pillars of the FAO plan of action to support the food and agriculture sector in addressing AMR

multidisciplinary and multi-sectoral collaboration. This is the basis of the FAO plan, which is bringing together internal production specialists, veterinarians, lawyers, food safety and laboratory specialists (among others). This multi-sectoral approach is further enhanced through FAO's tripartite working arrangements with WHO and OIE.

AMR AND FOOD

FAO recognizes that the food chain provides innumerable niches where microbial populations abound and in which AMR can emerge and be disseminated, and food plays a significant role in the very complex pathways of AMR transmission to humans. Pressures for the selection of AMR bacteria can occur in the food chain both in natural environments and following exposure to antimicrobials (including antibiotics, antivirals, fungicides and parasiticides) in food production, including aquaculture, livestock production and crop culture *(7, 10, 37, 43)*. It is also possible that exposure to sanitizers and biocides through the food chain can contribute to AMR *(34, 38)*. As with any other sector, awareness of the issue and the appropriate governance mechanisms or policy, as well as regulatory frameworks within which to operate, are key to meeting the AMR challenge in food production *(Fig. 2) (11)*. The two other major and interdependent aspects of management of the transmission of AMR via food are prudent use of antimicrobials in food production and management of the whole food chain to prevent or minimize the emergence and transmission of AMR and the need for monitoring and surveillance systems and the related laboratory infrastructure to provide an understanding of the current situation with regard to the levels of antimicrobial usage, the extent of resistance in the food chain, and the ability to monitor progress.

At the international level, the Codex Alimentarius has for over a decade recognized the importance of AMR in food safety and quality. The Codex Alimentarius, or "Food Code," was established by the FAO and the WHO in 1963 to develop harmonized international food standards that protect consumer health and promote fair practices in food trade. In 2005, Codex adopted its "Code of Practice to minimize and contain antimicrobial resistance" *(5)*, with the stated objective of minimizing the potential adverse impact on public health resulting from the use of antimicrobial agents in food-producing animals. It highlighted the importance of all those involved in *"the authorization, manufacture, sale, supply, prescription and use of antimicrobials in food-producing animals"* to *"act legally, responsibly and with the utmost care in order to limit the spread of resistant microorganisms among animals so as to protect the health of consumers."*

Figure 2. FAO action plan for addressing AMR focus areas and the related global action plan objectives

This is not an isolated standard. It is intended to be used in conjunction with Codex Guidelines on national regulatory programs associated with the use of veterinary drugs in food-producing animals (4). Subsequently, in 2011, Codex adopted "Guidelines for risk analysis of foodborne antimicrobial resistance" (5). This highlighted the importance that the international food regulatory community placed on having a structured and harmonized framework for AMR risk analysis. The guidelines also give a perspective on the complexity of the issue and the related challenges in addressing AMR within the food chain. Codex recently undertook work to review the extent to which countries are adopting and applying the existing Codex guidance and to identify major capacity development gaps and any other challenges countries face in adopting and applying these standards (6). This review, as well as developments in the area over the past 10 years, were considered by the Codex Alimentarius Commission in July 2016 in its decision to update some of the existing Codex texts and develop new texts on AMR. A new Codex task force on AMR was established, which will meet for the first time in 2017. However, given the importance of this issue and the need to provide countries with updated guidance in a timely manner, work has already begun with a first Codex working group on AMR, which was convened at the end of 2016. That group's tasks included detailed definition of the standard setting work to be undertaken by Codex as well as identification of the need for scientific advice related to AMR in the food chain, including risk management options for the containment of AMR, to support the standard setting process. In this context, the discussions on AMR in the food chain will continue within this international food safety forum in the coming years.

AMR IN FOOD CHAIN ECOSYSTEMS

Food chain ecosystems harbor foodborne pathogens, commensals and bacteria that together with bacteriophages provide a pool of genomes, or a microbiome, that may include genes encoding resistance to antimicrobials, or a resistome (10, 37, 43). The food chain microbiome is dynamic and can be the result of a co-mingling of those from domestic and wild animals, food, environmental systems and human systems. The diversity and level of AMR genes making up the resistome will be related to the bacterial flora typical of an ecosystem; the point in the food chain; the collective impact of food chain processes, practices and inputs; and the local environment, which varies among countries and regions. Bacteria present in the food chain can be intrinsically resistant strains and can be enriched or selected in a bacterial population following exposure to antimicrobials, possibly also sanitizers and biocides. Resistance may result from gene mutation or horizontal transfer of genes originating from the vast pool of resistance genes present in the entire food chain resistome (10, 37, 43).

It is well documented that antimicrobials used, appropriately or inappropriately, during primary production in agricultural systems have the potential to promote the emergence of AMR in foodborne pathogens, with the subsequent deleterious consequence of failed antimicrobial therapy for those foodborne illnesses for which antibiotic treatment is required for patients (8, 35, 37, 43). There is accumulating evidence that food may also be a source of extra-intestinal AMR infections, for example, as a source of urinary tract infections caused by common etiological agents such as extra-intestinal pathogenic *Escherichia coli*, where failure in clinical therapy is a public health issue (28). The consequences of exposure of consumers and food chain workers to AMR bacteria other than pathogens in food and to the food chain resistome generally is even more complex (43). Resistant microorganisms and AMR genes are ubiquitous in the environment around livestock production systems (45), in animal feeds (8) and in fertilized soils (32). Moreover, waterways and oceans are reservoirs for AMR genes (18), and there is evidence of recent exchange of antibiotic resistance genes between soil bacteria and clinical pathogens (16) and the jump of resistant organisms from human to livestock and from livestock to human (43). Co-mingling of various microbial ecosystems and resistomes occurs along the food chain, but the consequences of this cross-contamination and the risk of transmission of AMR via this means to the consumer is more speculative (10, 37, 43). An ecological and holistic approach is best suited to allow us to understand the risk of AMR emergence and transmission as well as the effectiveness of risk mitigations throughout the food chain from production to consumption. This will involve taking into consideration the overall resistome, the relationships of pathogens and commensals, and the quantitative effects of the environment, food processing and food handling on the risk (10, 25, 34, 37, 43, 45).

The food chain – a favorable niche for AMR transmission

Food ecosystems can provide ideal niches and conditions under which a food microbiome can be exposed to additional genetic material through interactions among animals, humans and the environment, and where bacterial genes can be mobilized. Transfer of AMR genes among food bacteria, including pathogens, has been demonstrated in vitro and in vivo, e.g., among lactic acid bacteria, between *Enterococcus* and *Listeria monocytogenes*, between *Salmonella* and *E. coli*, among *E. coli* and between isolates of species of animal and human origins (10, 37).

The food chain and food can provide ideal environments for the persistence and amplification of bacteria, and knowledge is accumulating of niches and scenarios that may favor AMR evolution and transmission. For example, bacteria usually do not reside in the ecosystem of a food or food environment as planktonic cells; rather they form assemblages or biofilms, free-floating or attached to surfaces, where

the same or multi-species communities are enclosed in a self-produced polymeric matrix *(25)*. Biofilms are found in animals and humans, on horticulture crops, in primary production environments and in water sources, and potentially on every non-sterile surface along the food chain *(17)*. While biofilms can offer a safe haven for its resident bacteria and protect them from antimicrobials, they may also be a niche for facilitation of AMR emergence and transmission *(25)*. In biofilms, bacteria are present in high density and in close contact; the matrix concentrates nutrients and compounds that facilitate cell interaction and protects the bacteria from biotic and abiotic exposures. Periodically bacteria-laden fragments slough off, disseminating contamination. Biofilms provide ideal protective structures that can enhance transfer of genetic elements, bacterial fitness and persistence and, in reverse, these elements may even promote biofilm formation.

Alexander Fleming was already aware of the potential risk of antimicrobial resistance, noting during his Nobel Prize speech that *"there is the danger, that the ignorant man may easily under dose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant"* *(15)*. He may not have realized the extent to which his observation would become a reality or the extent to which resistant organisms could emerge in non-clinical settings and become part of our food ecosystem. Exposure of bacteria to levels of antimicrobials exceeding the minimal inhibitory concentration (MIC) that inhibits susceptible strains results in the selection of resistant strains that can grow and outcompete susceptible strains; however, understanding the emergence of AMR bacteria is still evolving *(33)*. Increasing attention is being given to the potential for antimicrobial concentrations below the MIC, even several fold below, to result in the emergence of AMR bacteria that can subsequently be enriched in human, animal and environmental niches *(33)*. Low concentrations of antimicrobials can be present in various body tissues during and following human and veterinary therapy *(33)*. Likewise, the antimicrobial concentration within tissues can vary over the treatment period. Low levels of antimicrobials are found in the tissues of food animals treated therapeutically *(33)*, in environments (e.g., sewage, waste water, soils) *(1)* and in biofilms, as already described *(25)*. Antimicrobial residue levels in foods are generally considered to be reduced through the food chain, during food processing, and in the human gut to levels not considered hazardous to consumers. However, the effect of low residues levels on AMR among food microflora requires further investigation and understanding.

The transfer of AMR genes and the selection and enrichment of AMR bacteria in food systems is complex and can be influenced by the food matrix, environmental conditions, and the physiological state and growth fitness of a bacterium *(1, 37)*. Verraes et al. (2013) reviewed the mechanisms of AMR transmission and noted that a variety of food matrices have been shown experimentally to support the transfer of AMR encoding genetic material by conjugation and transformation among *Enterobacteriaceae* and lactic acid bacteria and in *Campylobacter jejuni* and *Bacillus* spp. *(37)*. Hurdles used for the control of growth of pathogens in food, e.g., low temperature and modified atmosphere packaging (MAP), may not necessarily prevent plasmid transfer among bacteria in food *(36)*. For example, plasmid transfer of AMR genes has been demonstrated experimentally between *Lactobacillus sakei* and *L. monocytogenes* in cooked ham at 7°C, given sufficient time and under MAP conditions *(36)*. Bacteria along the food chain can exist in varying physiological states and are subject to various stressors. Sub-lethal food preservation stresses (e.g., high or low temperature, osmotic and pH stress) have been shown to significantly alter the levels of phenotypic AMR, both positively and negatively, in pathogens such as *E. coli, Salmonella* Typhimurium, *Staphylococcus aureus* and *Cronobacter sakazakii* in food, and these changes may or may not be sustained after removal of the stress *(26, 37)*. This could have consequences in minimally processed foods.

Food reservoirs of bacteria and AMR elements

Except for commercially sterile foods, foods can be contaminated with a various bacterial species, and our knowledge is limited to those culturable in laboratories, which represents only a small portion of those present. Raw foods have the highest culturable bacterial concentrations, followed by minimally and fully processed foods, while bacteria can also be purposefully cultured in foods or included as pro-, pre- and synbiotics. Additional food contamination can occur when food is handled and as a consequence of environmental exposure, and the degree of contamination varies with the level of food chain sanitation and hygiene conditions. In FAO/WHO-supported studies of food chains in regions with poor hygiene management, more than half of raw chickens at markets were contaminated with $\geq 2 \log_{10}$ colony forming units (CFU) *E. coli*/ml rinsate; 75% of isolates were resistant to at least one antimicrobial tested, and some were multi-drug resistant *(29)*. Even with good hygiene conditions, ready-to-eat foods can be considered to be of satisfactory microbiological quality when contaminated with up to 10^4 CFU aerobic bacteria/g and this can include pathogens, e.g., a few *E. coli*/g or up to 100 coagulase-positive staphylococci/g, as well as others. Resistance to antimicrobials critical in human medicine and multi-resistance have been reported among opportunistic human pathogens isolated from foods and food processing environments, e.g., mesophilic and psychrotrophic *Pseudomonas* spp. isolated from goat and lamb slaughterhouse processing areas *(20)* and *Acinetobacter baumannii* on fresh vegetables collected from farmers' markets *(19)* and on commercial raw meat *(21)*.

Foods, including those of acceptable microbiological quality, could be considered as a virtual pool or sink of bacteria, bacteriophages, bacterial DNA and mobile genetic elements, some of which may include AMR genes *(10)*. A better understanding of the broader microbiome associated with a food and food chain environment and the genetic makeup of the resistome will help elucidate the role of the food chain in AMR emergence and transmission. With increasing use of new techniques such as whole genome sequencing and next generation sequencing, and with multiple ongoing efforts to sequence various food production biomes and resistomes, we should have a much better understanding of these aspects in the near future.

Known foodborne AMR pathogens

Foodborne pathogens, e.g., *Salmonella,* pathogenic *E. coli, Campylobacter, Staphylococcus* spp., *Enterococcus* spp. and extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria, have been the obvious focus of AMR attention. This is due to increased observance of AMR resistance among these bacterial groups and because links have been made between the presence of AMR strains of these bacteria in food, the use of antimicrobials in animal production, and human illness *(9, 20)*. This has placed emphasis on zoonotic foodborne bacteria and AMR in animal products, although the role of other commodities indirectly contaminated by these products and animal waste, or those possibly unrelated to animal product food chains, is less well understood and often not monitored. Microorganisms do not respect geographic borders, and global food trade pathways present ample opportunities for their global spread. For example, *Salmonella* DT104, which is typically resistant to five types of antibiotics [ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline], was first isolated in the United Kingdom in the 1980s and was later discovered to be endemic in cattle, which acted as a reservoir for the contamination of meat *(22)*. It spread worldwide with alarming speed during the 1990s and is now common, especially in Europe and North America. Of particular and ongoing concern is the fact that it has shown an ability to acquire resistance to other types of antibiotics, including the clinically important fluoroquinolones and cephalosporins. A recent genomics study investigating the emergence and spread of *Salmonella* DT104 suggests that this organism may have first appeared in approximately 1948 and that multiple transmission events led to its global spread, highlighting the complexity of AMR in the food chain *(22)*.

Reports of pathogens and commensals with variant AMR patterns or enhanced resistance not previously seen are continually occurring. *E. coli* O104:H4, which caused a major outbreak of foodborne disease in Germany in 2011 (from sprouted seeds) and resulted in 53 deaths and 2,987 illnesses, was found to be resistant to a number of antibiotics [ampicillin, trimethoprim, cephalosporins and tetracycline] *(2)*. Furthermore, it possessed a plasmid-borne gene for ESBL production, which can mean resistance to a wide range of therapeutic antibiotics *(27)*. *Campylobacter jejuni* that have variant AMR genes and are unusually potent in the efflux of antibiotics, resulting in enhanced resistance to multiple antibiotics such as florfenicol, and fluoroquinolones (used in clinical treatment of campylobacteriosis), have been reported recently *(44)*. The AMR mutant strains were detected in chickens and pigs in China and were believed to have resulted from the selective pressure of frequent antibiotic use and to have spread by horizontal gene transfer. The first report of detection of plasmid-mediated colistin resistance in commensal *E. coli* isolated from food animals in China in 2011 caused international alarm as resistance to the polymixin group of antibiotics had previously been associated with chromosomal mutations and not horizontal gene transfer, and this antibiotic has become a drug of last choice for some clinical infections *(23)*. Further cross-resistance to colistin has been reported in clinical *Klebsiella pneumoniae* strains without loss of fitness or virulence following exposure to the antiseptic chlorhexidine used for a variety of clinical purposes, such as equipment maintenance, mouthwashes, skin antisepsis, and wound dressings *(38)*.

As even these recognized pathogens may present unexpected challenges, surveillance and monitoring of pathogens and AMR in human patients and non-human sources is required to identify and respond to threats in a timely manner. For example, an epidemic of invasive (compared with the usual non-invasive) non-typhoidal salmonellosis is currently occurring among high risk individuals in regions of Africa and parts of Europe *(3)*. The systemic clinical manifestations indicate antimicrobial treatment, and AMR among the strains is becoming a major concern. Person-to-person transmission appears common; however, if the traditional role of food, water and animals in transmission occurs, the epidemic could take a dramatically different path.

CONCLUSIONS

AMR has been recognized by world leaders as a threat to global public health, comparable with HIV, noncommunicable diseases, and Ebola, that requires a multi-sectorial commitment at the international level for its control. The FAO, WHO and OIE have developed collaborative global development plans in which food is recognized as having a role in the transmission of AMR, and interventions have been proposed. The Codex Alimentarius recognized this issue more than a decade ago and developed a Code of Practice and AMR risk analysis Guidelines. It is timely that Codex is now updating these standards and taking on new work to address gaps in food chain AMR management.

The emergence of AMR in zoonotic foodborne pathogens and indicator bacteria associated with the imprudent use of antimicrobials in animal production is well known, and control of use of antimicrobials at the food production level is an essential intervention for preventing the transmission of AMR bacteria in animal-derived foods. However, as food passes along the food chain to the consumer, it will be exposed to and carry a diverse and dynamic bacterial flora and may be further exposed to antimicrobials such as sanitizers and biocides. Foodborne pathogens, commensals, and other bacterial contaminants can potentially carry AMR genetic elements that will contribute to the food chain resistome, and the risk posed by the resistome and AMR bacteria other than AMR foodborne pathogens in a food at the point of consumption is less well understood. The food chain ecosystem can provide niches and conditions for gene transfer and for selection and persistence of AMR bacteria as has been demonstrated experimentally in foods. A better understanding of the role of food in AMR transmission, together with an understanding of the potential associations between AMR genes and other important genes encoding virulence and the survival and persistence of pathogens in foods and their environments, is required.

Observations of the transmission of AMR by foods emphasizes the ongoing need for controlling the presence of foodborne pathogens in foods as well as the transmission of the resistome present in the bacterial flora of foods. Basic sanitation and hygiene are prerequisites for hazard control in foods and now should be seen as an essential requirement for the control of AMR transmission as well.

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