Food and Agriculture Organization of the United Nations

DRIVERS, DYNAMICS AND EPIDEMIOLOGY OF ANTIMICROBIAL RESISTANCE IN ANIMAL PRODUCTION

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EXECUTIVE SUMMARY

It is now accepted that increased antimicrobial resistance (AMR) in bacteria affecting humans and animals in recent decades is primarily influenced by an increase in usage of antimicrobials for a variety of purposes, including therapeutic and non-therapeutic uses in animal production. Antimicrobial resistance is an ancient and naturally occurring phenomenon in bacteria. But the use of antimicrobial drugs – in health care, agriculture or industrial settings – exerts a selection pressure which can favour the survival of resistant strains (or genes) over susceptible ones, leading to a relative increase in resistant bacteria within microbial communities. It has been observed that, in countries where use of particular substances (e.g. fluoroquinolones) is banned in animal production, there are low levels of resistance to these antimicrobials in livestock populations. The rate of AMR emergence in ecosystems such as the human or animal gut is likely to be highly dependent on the quantity of antimicrobials used, along with the duration and frequency of exposure. In animal production, the prolonged use of antimicrobial growth promoters (AGPs) at subtherapeutic levels in large groups of livestock is known to encourage resistance emergence, and is still common practice in many countries today. Due to the interdependence and interconnectedness of epidemiological pathways between humans, animals and the environment, determining the relative importance of factors influencing AMR emergence and spread in animal production is a significant challenge, and is likely to remain one for some time.

In intensive livestock production systems, resistant bacteria can spread easily between animals and this can be exacerbated if biosecurity is inadequate. While some studies have shown reduced levels of AMR on organic farms, a high prevalence of multidrug-resistant (MDR) *Campylobacter* strains has been detected in organic pig farms in the United States even in the absence of antimicrobial usage (AMU).

In aquaculture, AMR can develop in aquatic and fish gut bacteria as a result of antimicrobial therapy or contamination of the aquatic environment with human or animal waste. The extent and persistence of antimicrobial residues in aquatic systems is unknown and current evidence is conflicting. Furthermore, no international guidelines currently exist for maximum antimicrobial residue limits in water. Water is an important vehicle for the spread of both antimicrobial residues and resistance determinants, since contaminated water can be consumed directly by humans and livestock and used to irrigate crops.

Food is likely to be quantitatively the most important potential transmission pathway from livestock to humans, although direct evidence linking AMR emergence in humans to food consumption is lacking. There is a theoretical risk of widespread dissemination of AMR due to the increasingly global nature of food trade and human travel. This would mean that strains of resistant bacteria could now very quickly reach parts of the world where they had previously not been present. Agricultural systems in emerging economies such as China and India have changed radically in recent years, becoming increasingly intensive in order to meet growing domestic and global demands for animal protein. This is likely to heighten the occurrence and spread of infectious diseases in these systems, thereby leading to increased AMU and therefore resistance.

If the selection pressure resulting from AMU in animals and humans were to be removed, this would still not completely halt the emergence and global spread of AMR due to the ability of AMR genes to move between bacteria, hosts and environments, and the occurrence of spontaneous mutations.

However, the release of large quantities of antimicrobials or resistant bacteria into the environment is still thought to be an important point for control, and therefore measures which encourage the prudent use of antimicrobials are likely to be extremely useful in reducing the emergence and spread of AMR. Future development of quickly biodegradable antimicrobials could help to reduce environmental contamination, and pharmacodynamic studies in livestock can be used to inform the optimization of AMU. Improved hygiene and biosecurity should be a major focus for all types of animal production systems so that the risks of introducing pathogens and resistance genes – and the spread of these within animal populations – can be reduced. Detailed, specific recommendations for countries to move towards more prudent AMU in different agricultural settings are, however, beyond the scope of this paper.

An improved understanding of the epidemiology of AMR emergence and spread in animal production will provide an essential foundation for successful mitigation strategies. There are still considerable gaps in our understanding of the complex mechanisms that lead to the emergence of AMR in bacteria, and the interactions that take place within microbial ecosystems enabling the transfer of resistance between bacteria. There are insufficient data at present to determine quantitatively how important the selection pressure of AMU is for the emergence of AMR in bacteria. Evidence regarding AMR transmission pathways between food animals and humans is lacking, especially from low- and middle-income countries (LMICs).

Such pathways are likely to be highly complex and multi-directional, especially in LMICs, but are still largely unknown. There remains little doubt, however, that the most significant factor in AMR emergence in humans is AMU for human treatment and prevention. It is clear that both human and animal AMU can contribute to environmental contamination, although collection of meaningful data is challenging. The relationships between different types of farming systems and both AMU and the emergence and spread of AMR are discussed in this paper, including extensive and organic systems, but there is still a notable lack of knowledge on the role that sustainable agriculture systems can play in combatting AMR. Most importantly, future research needs to involve an interdisciplinary (e.g. One Health) approach, integrating agricultural, medical, environmental and social sciences, and especially recognizing the importance of human behaviour. A set of specific recommendations to fill current knowledge gaps is presented in the final section of this technical paper.

INTRODUCTION

Antimicrobial resistance (AMR)[1] both in human and veterinary medicine has reached alarming levels in most parts of the world and has now been recognized as a significant emerging threat to global public health and food security. In June 2015, the Food and Agriculture Organization of the United Nations (FAO) passed a resolution on AMR at its governing Conference. This followed the adoption of counterpart resolutions on AMR by The World Organisation for Animal Health (OIE) and the World Health Organization (WHO) in May 2015[2], and marked the beginning of a joint effort by the three organizations to combat AMR globally. The present technical paper was commissioned by FAO and is intended to inform a technical audience comprising scientists, policy-makers and stakeholders (including veterinarians and medics) in FAO Member States. A review was undertaken of the available scientific literature, grey literature, reports, and other sources of evidence, to examine the current state of knowledge on the relationship between animal production and AMR emergence and spread. The review methodology is described in detail in Appendix 1.

Overuse of antimicrobials and improper use in many parts of the world are recognized as key drivers of the emergence and spread of AMR (Aminov and Mackie, 2007, APUA, 2008, Aarestrup *et al.*, 2008, Acar and Moulin, 2012). Antimicrobials are used in food animals for treatment and for non-therapeutic purposes, and play a critical role in saving lives in both humans and animals. Over the last decade, global livestock production has been growing rapidly and has moved increasingly towards industrialized systems where antimicrobial use (AMU) is an integral part of production. It is projected that two thirds of the future growth of AMU will be for animal production (Van Boeckel *et al.*, 2015). Although AMU in animals for growth promotion, prophylaxis and metaphylaxis (i.e. medicating mixed groups of healthy and infected animals in order to control outbreaks of disease) has been substantially reduced in high-income countries in recent years, data available indicate that livestock AMU will continue to increase in low- and middle-income countries during the next decades due to the growing demand in LMICs for animal protein (Van Boeckel *et al.,* 2015).

Consequently, there is likely to be a commensurate increase in resistance to commonly used antimicrobials in these countries and regions, which does not bode well for treatment and management of infections in both humans and animals. This is especially important for zoonotic pathogens but also for commensal bacteria as these can act as reservoirs for resistance genes within the gut microbiota and the environment (the "resistome") (APUA, 2008). Indeed, resistance to colistin, an antimicrobial used as a last resort for treating multidrug-resistant (MDR) infections in humans, was recently detected in an-

[1] The term antimicrobial resistance (AMR) is used to refer to the ability of any microorganism (bacteria, viruses, parasites and fungi) to withstand the effect of one or more antimicrobial agents at clinically attainable concentrations, usually resulting in therapeutic failure. Throughout this document, AMR will be used to include resistance to antibacterial, antiviral and antiparasitic agents, although the focus will primarily be on bacterial resistance to antibacterial agents.

[2] Details of all three resolutions on AMR are now available in the public domain:
FAO resolution: *http://www.fao.org/3/a-mm736rev1e.pdf*
OIE resolution: *http://www.oie.int/en/about-us/key-texts/resolutions-and-recommendations/resolutions-adopted-by-the-oie-international-committee/*
2015 WHO resolution: *http://apps.who.int/gb/ebwha/pdf_files/WHA68/A68_ACONF1Rev1-en.pdf*

imals, retail meat and humans in China and subsequently has been discovered in most world regions (Skov and Monnet, 2016).

Despite the public health significance of, and global attention to, AMR, a number of important questions are still surrounded by significant uncertainty, especially concerning the epidemiological relationships between AMU and food animals, the occurrence of AMR in food animals and the exposure of humans to AMR via food products. This technical paper deals with the epidemiology of the emergence of AMR as a consequence of AMU in animal production, and the risk of its spread via food distribution and the environment. While this paper aims to take a global perspective, there are data gaps in certain regions of the world which means that some of the information presented has a European bias. The discussion begins with a technical description of the current state of knowledge regarding the acquisition of AMR by bacteria, and types and mechanisms of resistance in bacteria. Subsequently, the influence of animal production on the emergence of AMR in animals and humans is discussed. This is followed by an overview of local and global pathways of AMR transmission, and how these may be influenced by different livestock production systems.

THE EMERGENCE OF ANTIMICROBIAL RESISTANCE IN BACTERIA

Antimicrobial resistance was first described in 1940 in *Bacillus coli* (now known as *Escherichia coli*) by Abraham and Chain (1940), shortly before the start of the use of penicillin to treat infectious diseases in humans in the same year (Chain *et al.*, 1940) and not long after its discovery by Fleming (1929). Since most antimicrobials in clinical use are naturally produced by soil microorganisms, such microorganisms are the source of many resistance genes now found in clinically relevant bacteria, as was demonstrated more than 40 years ago (Benveniste and Davies, 1973). Further phylogenetic analysis has shed some light on the evolutionary origins of resistance, indicating that bacteria evolved AMR genes long before the "antibiotic era" (Finley *et al.*, 2013, Aminov and Mackie, 2007, Wellington *et al.*, 2013, Martinez and Baquero, 2009), and even developed defences against synthetic compounds (D'Costa *et al.*, 2011). There is growing evidence that AMR is in fact an ancient and natural part of the genome of environmental bacteria (Bhullar *et al.*, 2012). However, it is important to realize that AMR was very rare in clinical isolates predating the introduction of antibiotics, as demonstrated in a retrospective analysis by Hughes and Datta (1983), which provides strong evidence for the central role of AMU in the emergence and spread of AMR as a public health threat.

EVOLUTION OF RESISTANCE GENES

In natural ecosystems, expression of AMR genes can act as a defence mechanism against antimicrobial- or toxin-producing competitors in the same ecological niche, or as a self-preservation mechanism in antimicrobial-producing bacteria (Martinez and Baquero, 2009, Courvalin, 2008).

However, as the role of antimicrobials both in bacterial physiology and microbial ecology is mostly unknown – with theories ranging from the regulation of cell growth mobilization (Amábile-Cuevas, 1993, Davies and Davies, 2010) to environmental signalling (Yim *et al.*, 2007) – the role and evolutionary origins of AMR genes remain an educated guess.

Bacteria that are able to metabolize antimicrobials and use these as a source of nutrients have been found to express multidrug resistance (APUA, 2008). It is likely that resistance genes and determinants from these bacteria can be transferred to other bacterial species, even taxonomically and genetically distant ones (Aminov and Mackie, 2007). Many resistance genes were originally used by bacteria to support vital metabolic processes (Aminov and Mackie, 2007, Martinez and Baquero, 2009, Martinez, 2008). For example, some signalling molecules produced by environmental bacteria for communication purposes have been found to have antimicrobial activity (Martinez, 2008, Martinez and Baquero, 2009).

β-lactamase enzymes encoded by plasmids in environmental bacteria may originally have been involved in synthesis of peptidoglycans rather than in providing resistance to β-lactam antimicrobials (Martinez and Baquero, 2009). Environmental soil and water bacteria have been found to carry a pool of resistance genes (the "resistome") which can act as a reservoir of resistance for human pathogens (Forsberg *et al.*, 2012, Lupo *et al.,* 2012, APUA, 2008).

Environmental changes – such as those induced by anthropogenic activities (e.g. use of antimicrobials) – increasing human populations, urbanization,

lack of treatment of sewage and animal waste (Martinez, 2008), and the intensification of agriculture and industry, can affect the emergence of resistance in bacterial populations (IFT, 2006, Li *et al.*, 2015). The transfer of resistance genes between humans, animals and the environment has recently been reported in low-income population settings in Latin America (Pehrsson *et al.*, 2016). Increased contact between human settlements, food-producing animals and wildlife has been reported as an important factor in the transfer of resistance traits and bacteria to species that usually would not be naturally exposed directly to selection pressure through antimicrobial therapy (Cristobal-Azkarate *et al.*, 2014, Österblad *et al.*, 2001). Horizontal gene transfer (HGT) movements between farm environments, food, and human gut microbiota were estimated in one study to be composed of over 75 percent resistance genes, but this was found also true for HGT episodes involving the human skin and oral system (Smillie *et al.*, 2011).

HGT movements were most likely to occur between phylogenetically diverse bacteria sharing the same ecological niche (e.g. human gut flora). The horizontal movement of genes can occur between Gram-negatives, Gram-positives and Actinobacteria; between aerobes and anaerobes; and between non-pathogenic and human-, animal- or plant-pathogenic bacteria (Amábile-Cuevas and Chicurel, 1992). For instance, glycopeptide-producing bacteria in the environment have been identified as a potential source of genes encoding vancomycin-resistance (*van* genes) to enterococci bacteria that can cause opportunistic disease in humans (i.e. vancomycin-resistant enterococci or VRE) (Courvalin, 2008). Enterococci can acquire, maintain and disseminate resistance genes to other enterococci and Gram-positive bacteria through mobile genetic units (e.g. transposons, plasmids). In some instances, mobile genetic units can account for up to 38 percent of the genome of enterococci (Werner *et al.*, 2013). Furthermore, it has been observed that enterococci of animal origin can also colonize the human gut (Werner *et al.*, 2013).

Therefore, dissemination of resistance genes can occur clonally, through vertical spread, and also via horizontal transfer through transposons and integrons (intracellular gene mobilization) and through integrative and conjugative elements (ICEs, such as conjugative transposons) and plasmids, among others (Amábile-Cuevas, 2012, Courvalin, 2008).

Resistance genes acquired through horizontal transfer and mutations can provide bacteria with an evolutionary advantage in relation to other competitors in the same ecological niche, as long as the resistance does not result in a negative impact on the bacteria's physiology, also known as the "fitness cost" (Martinez and Baquero, 2009, Courvalin, 2008). A particular population of bacteria may lose resistance traits in the absence of selection pressure by antimicrobials (Courvalin, 2008). However, acquired resistance genes may indirectly provide an evolutionary advantage for the bacteria, even in the absence of selection pressure (Aminov and Mackie, 2007). A bacterium may also undergo further "compensatory mutations" that allow it to reduce the fitness cost of the acquired resistance genes (Aminov and Mackie, 2007, Bergstrom and Feldgarden, 2007, IFT, 2006).

The presence of antimicrobials in the environment – as observed in hospitals or intensive farm settings – has been associated with the survival of strains with higher rates of mutation (e.g. bacteria with hypermutator phenotypes) (Martinez and Baquero, 2009, Courvalin, 2008, Aminov and Mackie, 2007). Hypermutator phenotypes have been observed in chronic infections in humans (Martinez and Baquero, 2009). Gullberg *et al*. (2011) have observed that very low concentrations of antimicrobials could enhance the survival of gene mutations in a bacterial population. Genetically, some bacteria have evolved to be diploids, which allows them to

express both susceptibility and resistant traits (Courvalin, 2008). Likewise, mechanisms that previously were used for other purposes (e.g. efflux pumps in cell membranes) can be adopted by bacteria – such as observed in resistant *Escherichia coli* strains (Webber and Piddock, 2003) – in order to survive in an adverse environment, even in the presence of semi-synthetic or synthetic antimicrobials to which bacterial populations have not been previously exposed (e.g. fluoroquinolones) (Martinez and Baquero, 2009 Aminov and Mackie, 2007, Courvalin, 2008).

TYPES OF RESISTANCE: INTRINSIC VERSUS ACQUIRED

Bacteria can be naturally resistant to certain antimicrobial groups or substances (Prescott, 2008) (intrinsic resistance), or they can obtain resistance to antimicrobials through a variety of mechanisms, such as mutation (acquired resistance).

A brief overview of intrinsic and acquired resistance mechanisms is given in Table 1 and discussed below.

Intrinsic resistance

Intrinsic resistance is mediated by chromosomal genes (Alekshun and Levy, 2007, Courvalin, 2008) and is usually linked to physiological or anatomical characteristics of the bacteria (IFT, 2006), hence it is usually a trait shared by all organisms within the same genus or species (Courvalin, 2008). Resistance to penicillin G expressed by most Gram-negative bacteria is a common example (Boerlin and White, 2013, SCENIHR, 2009), this is due to the complexity of its cell wall with the presence of an outer membrane – absent in Gram-positive bacteria (IFT, 2006).

Acquired resistance

Vertical transmission. Chromosomal mutations are extremely rare (i.e. 10^{-7} to 10^{-9} frequency), but are very relevant to the development of resistance in bacterial clones (Courvalin, 2008) (Table 1). Mutations can either affect target or regulatory genes (Courvalin, 2008). Target mutations occur in structural genes that encode the specific targets of antimicrobial action (Courvalin, 2008).

Single point mutations are the most commonly observed once an antimicrobial substance is introduced (Bergstrom and Feldgarden, 2007), such as that observed with quinolone and macrolide resistance in *Campylobacter* spp. (Aarestrup *et al.*, 2008, Moore *et al.*, 2006, Cambau and Guillard, 2012).

Regulatory mutations usually affect gene expression mechanisms and are difficult to predict as they can occur spontaneously (Courvalin, 2008).

TABLE 1. Types of resistance observed in bacteria

	Intrinsic resistance	Acquired resistance
Definition	• Natural traits • Species or genus specific	• A strain that develops resistance to an antimicrobial to which it was previously susceptible • Present only in certain strains of a species or genus
Mechanisms of resistance acquisition	• Inherent structural or functional characteristics of the bacteria that allow it to tolerate or be insensitive to an antimicrobial substance or class	Vertical transmission • Spontaneous gene mutation • Induced gene mutation Horizontal gene mutation • Bacterial transformation • Bacterial transduction • Bacterial conjugation

Source: Boerlin and White, 2013

Unspecific efflux pumps, encoded at chromosomal level and therefore genus-specific, can confer multidrug resistance to unrelated antimicrobial substances (Courvalin, 2008, Demple and Amábile-Cuevas, 2003).

Horizontal transmission. When genes from a cell are transferred into another cell, independently of a reproductive event, this is known as "horizontal gene transfer" (HGT) (Table 1). HGT occurs through three main mechanisms: (a) transformation, the uptake of free DNA by a "competent" bacterial cell; (b) transduction, the mobilization of bacterial DNA from one bacterial cell to another by a bacteriophage (i.e. a virus); and (c) conjugation, the mobilization of DNA from a donor bacterium to a recipient bacterium, requiring physical contact and conjugative machinery (Amábile-Cuevas and Chicurel, 1992, Amábile-Cuevas, 2012).

HGT is probably the most relevant mode of resistance emergence and spread in bacterial populations (Aarestrup *et al.*, 2008). Horizontal passage of resistance can arise through the transfer of single resistance determinants or of combinations of genes inserted in mobile structures: gene cassettes incorporated into integrons, which can be incorporated into transposons, and transposons which can be integrated into plasmids (Martinez and Baquero, 2009, Amábile-Cuevas and Chicurel, 1992). Integration and transposition allows the intracellular movement of genes, gathering several resistance determinants into a single genetic element, and also rearranging genes to modify their expression (Amábile-Cuevas, 2012, Mathew *et al.*, 2007, Levy and Marshall, 2004, Mazel, 2004). Integrons were initially identified in Gram-negative bacteria but have also been detected in Gram-positives (Levy and Marshall, 2004). Class I integrons are commonly associated with resistance and found in isolates from livestock (Mathew *et al.*, 2007), the presence of class I integrons in *Escherichia coli* is very dependent on selection pressures of human origin (Díaz-Mejía *et al.*, 2008), showing that antimicrobial pressure does not only select for AMR traits, but also for mechanisms of mobilization. Transposons, in turn, facilitate the transfer of genetic material within the same or different DNA molecules or even between different organisms as ICEs, as previously described (Martínez *et al.*, 2007). Plasmids are DNA structures that can be transmitted horizontally and/or vertically through bacterial clones (Martinez and Baquero, 2009). However, not all mobile modular units are effectively transferred or expressed between bacteria (Aarestrup *et al.*, 2008). For instance, some Gram-positive bacteria are not able to express genes transferred from Gram-negative bacteria (Courvalin, 2008).

Gathering of resistance genes in a single genetic element enables the co-selection of resistance by unrelated antimicrobials leading to multidrug resistance, and even potentially by non-antimicrobial compounds such as metal ions and biocides. Also, the assembly of plasmids enables the acquisition of resistance to several unrelated antimicrobials through a single event, such as conjugation. Finally, resistance genes are often found along with virulence traits in the same genetic element, making the bearer of such an element an enhanced, multidrug-resistant pathogen (Amábile-Cuevas, 2003).

Adaptive resistance

A number of regulated responses to environmental stress can activate AMR phenotypes by means of active efflux and/or diminished permeability. Among the best characterized of these responses are the *mar*RAB regulon and the *sox*RS regulon.

Both were first described in *Escherichia coli* but there are equivalent systems in many Gram-negative bacteria (Demple and Amábile-Cuevas, 2003).

Activating agents include a variety of compounds, ranging from antimicrobials to non-antibacterial drugs (e.g. phenazopyridine) (Amábile-Cuevas and Arredondo-García, 2013), and herbicides (e.g. glyphosate) (Kurenbach *et al.*, 2015). Resistance to antimicrobials achieved through these mechanisms

disappears when the stimulus is gone and is thus distinct from intrinsic resistance. Single mutations in regulatory genes can cause a permanent overexpression of the whole regulon, hence turning the mutant into a full-resistance phenotype. However, there is no evidence for such mutations having been horizontally mobilized, confining this kind of acquired resistance to vertical inheritance.

MECHANISMS OF BACTERIAL RESISTANCE

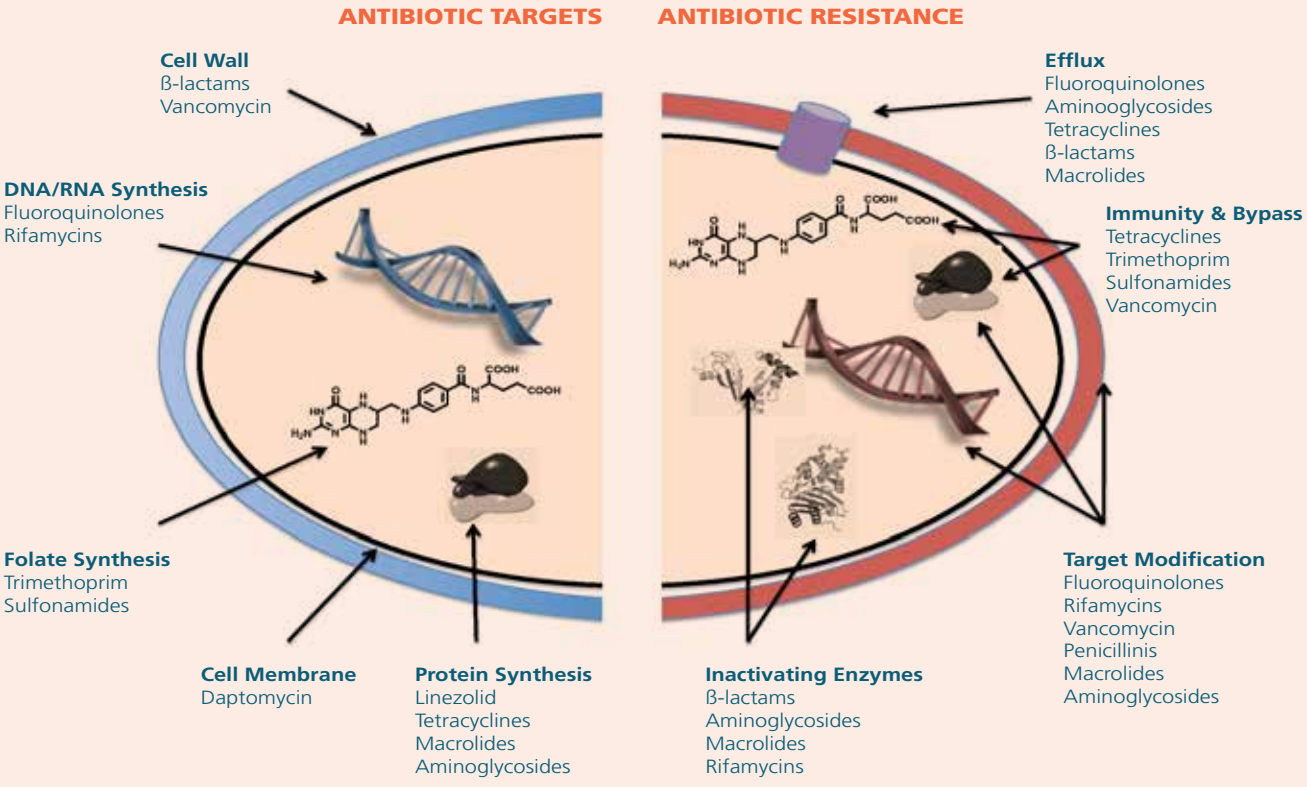
There are a number of mechanisms that render a bacterial cell resistant to one or several antimicrobials.

These mechanisms can be organized into five broad categories: (1) decreased accumulation of the antimicrobial within the cell, either through diminished permeability and/or active efflux of the antimicrobial from the bacterial cell; (2) enzymatic modification or degradation of the antimicrobial; (3) acquisition of alternative metabolic pathways to those inhibited by the antimicrobial; (4) modification or protection of the antimicrobial target; and (5) overproduction of the target enzyme (*van* Hoek *et al.*, 2011). The cellular targets of antibiotics, and bacterial resistance mechanisms to main antimicrobial groups, are shown in Figure 1. A brief summary of acquired resistance mechanisms for different antimicrobial groups is provided in Appendix 2.

Multidrug resistance

A bacterial cell can achieve resistance to multiple, unrelated antimicrobials, by means of a single mutation. Such mutations often involve decreased

FIGURE 1. Antibiotic targets and mechanisms of resistance



Source: Wright, 2010

accumulation of antimicrobials in the cell, either by decreased permeability (e.g. reduction in the number and/or pore size of outer membrane porins (Omps) in Gram-negative bacteria), and/or increased efflux through unspecific pumps (e.g. AcrAB in enteric bacteria, Mex in *Pseudomonas* spp.).

Other biocides and toxic agents

A number of genes encoding resistance to non-antimicrobial agents have been found linked to AMR genes in single genetic elements, fostering co-selection. For instance, mercury-resistance genes *mer* (encoding transport systems, reductase enzymes and even lyase enzyme to detach mercury from organomercurial compounds) are commonly found along with AMR genes in the Gram-negative transposon Tn*21*, which also harbours an integron and staphylococcal resistance plasmids. Likewise, *qac* genes mediating resistance to quaternary-ammonium disinfectants – commonly used in hospital and agriculture settings – through active efflux, are found in the conserved region of class I integrons (Liebert *et al.*, 1999).

THE RELEVANCE OF ANIMAL PRODUCTION IN THE EMERGENCE OF ANTIMICROBIAL RESISTANCE

There is a substantial body of evidence to support the view that the emergence of antimicrobial resistance in bacteria in livestock populations is connected to the emergence of AMR in bacterial populations that colonize and infect humans (Singer *et al.*, 2003, ECDC/EFSA/EMA, 2015, O'Neill, 2015). For example, a recent systematic review found that "a proportion of human extra-intestinal expanded-spectrum cephalosporin-resistant *Escherichia coli* (ESCR-EC) infections originate from food- producing animals", with poultry as a probable source (Lazarus *et al.*, 2015).

Despite this, other recent studies claim that most of the emergence of AMR in bacteria in humans appears to originate from AMU in humans, while the majority of AMR bacteria in livestock seem to originate from AMU in livestock. For example, phylogenetic and whole genome sequence analysis of *Salmonella enterica serovar Typhimurium* DT104 in human and livestock populations in Scotland has shown a greater diversity of AMR genes in human *S. Typhimurium* DT104, by comparison with those isolated in local livestock populations. The implication is that there were contributing sources other than foods of animal origin or livestock (Mather *et al.*, 2013). Studies based on the phenotypes of AMR bacterial populations have yielded similar conclusions (Mather *et al.*, 2012). In addition, a recent systematic review reported that usage of antimicrobials of critical importance for human treatment (such as fluoroquinolones and third- and fourth-generation cephalosporins) was higher in humans than in food-producing animals after adjusting by biomass (ECDC/EFSA/EMA, 2015). In most cases, in both animals and humans, a positive association was found between the volume of antimicrobial consumption and prevalence of resistance in the exposed bacterial populations. Nevertheless, there is consensus within the scientific literature that there are routes for spillover of AMR between the bacterial populations of humans and food-producing animals in both directions. The most commonly mentioned route is via AMR bacteria (and genetic material) passed through food distribution and consumption, the majority of which are colonists of the host gastrointestinal tract (Lazarus *et al.*, 2015). Such bacteria may be commensal in animals but pathogenic in humans, or may be commensal in both, but may later convey resistance to food-borne pathogens in the human gut (Singer *et al.*, 2003). In general, the repercussions of such crossover events, in terms of human disease, appear to be exhibited in outbreak form (Mather *et al.*, 2013), though this apparent pattern may be a result of reporting bias, as a result of a relatively high abundance of research into this route and evidence generated through government-led outbreak investigations. Strong and direct evidence for AMR transmission via food is still limited (Lazarus *et al.*, 2015). For instance, a study in The Netherlands reported increased levels of ESBL (extended spectrum beta-lactamase) enzyme-producing bacterial isolates with similar resistance genes in poultry meat and humans (Overdevest *et al.*, 2011). Further, there is evidence of AMR occurrence not only in animal-derived foodstuffs (Raufu *et al.*, 2014, Dipeolu and Alonge 2002, Muriuki *et al.*, 2001, Kariuki *et al.*, 2013, NARMS, 2013, ECDC/EFSA/EMA, 2015, Duong *et al.*, 2006, Thai *et al.*, 2012) but also in vegetables (de Vasconcelos Byrne *et al.*, 2016, Kim *et al.*, 2015, McGowan *et al.*, 2006). The recent detection of resistance to colistin in food-borne pathogens in humans, livestock, meat and vegetables across different countries raises the issue of the

potential role of global travel and trade in the transboundary dissemination of resistance genes (Skov and Monnet, 2016, Doumith *et al.*, 2016, Liu *et al.*, 2016, Kluytmans–van den Bergh *et al.*, 2016, Zurfuh *et al.*, 2016).

Some of the resistant bacterial populations documented, which are of importance to human health, are shown in Table 2. This is not an exhaustive list.

DRIVERS OF AMR EMERGENCE IN ANIMAL PRODUCTION

Although antimicrobial resistance occurs primarily as a consequence of selection pressure placed on susceptible microbes by the use of antimicrobial agents (Dione *et al.*, 2009, Glynn *et al.*, 2004, Grace *et al.*, 2008, Koningstein *et al.*, 2010), a variety of other factors also contribute to the emergence and spread of resistance. This section will specifically focus on factors driving AMR in agriculture.

Measures such as vaccination, limited co-mingling, adequate ventilation and temperature controls, biosecurity, appropriate nutrition and housing, and quality-assurance programmes are commonly used in modern animal production to reduce the risk of introduction and spread of infections in herds. But it must be recognized that these risk-management practices usually require substantial financial investment, as well as training and incentivizing staff. Even if these measures are implemented properly, however, a residual disease risk will remain (Adelaide *et al.*, 2008, Cerniglia and Kotarski, 2005, Kariuki *et al.*, 2013). Consequently, antimicrobials are commonly used non-therapeutically in livestock production as a kind of "insurance" in addition to other animal disease risk-management measures.

Resistance to tetracycline, penicillins and sulphonamides has been commonly observed among chicken and swine bacterial isolates, and MDR has been reported as significantly higher in these isolates than those from cattle. The intensive conditions under which pigs and chickens are often housed may be associated with greater disease potential and therefore a greater AMU in order to

TABLE 2. Sources of antimicrobial resistance (bacteria and bacterial genes) in animal production settings

Bacterial species	Antimicrobial resistance pattern	Infections commonly observed in humans	Animal sources of human infection	Other known sources of human infection
***Campylobacter* spp.**	Fluoroquinolones	Gastrointestinal *(sequelae: Guillain-Barré syndrome)*	Food-producing animals (poultry)	Raw unpasteurized milk, water
***Enterococcus* spp.**	Aminoglycosides Ampicillin Vancomycin		Food-producing animals (poultry)	
***Escherichia* coli**	Quinolones Sulphonamides Trimethoprim	Gastrointestinal, UTI, HUS		
LA-MRSA			Food-producing animals (pigs, calves, cows)	
***Salmonella* spp.** (non-typhoidal)	Cephalosporins Quinolones Tetracyclines	Gastrointestinal	Food-producing animals (pigs, cows, poultry)	

Legend: HUS - Hemolytic Uremic Syndrome, LA-MRSA - Large Animal Methicillin-resistant *Staphylococcus aureus*, UTI - Urinary Tract Infection
Source: Adapted from Furuya and Lowy (2006)

control sub-clinical infections (Duff and Galyean, 2007). In some non-European countries, antimicrobials are widely used by farmers without veterinary supervision due to their relatively low cost and ready availability for sale over the counter (Laxminarayan *et al.*, 2013). In Kenya, tetracyclines account for 55 percent of the antimicrobials used in food animals (Mitema *et al.*, 2001).

In another study in Kenya by Kariuki *et al.* (2013), oxytetracycline was the most commonly used among small-scale poultry farmers, while other antimicrobials used included fluoroquinolones (norfloxacin and enrofloxacin), erythromycin, sulphonamides and co-trimoxazole. Antimicrobials were readily available and mostly purchased over the counter or from animal health assistants, without resorting to veterinary advice. Drug quality was identified as an issue, as approximately one third of the drugs failed quality tests carried out by the National Quality Control Laboratory. In the same study, overall AMR among the pathogens and indicators tested was highest in poultry isolates, followed by those from pigs and cattle. This probably reflects the more intensive nature of poultry farming and higher levels of AMU observed.

Enteric bacterial isolates detected in food-producing animals and meat were commonly resistant to ampicillin, tetracycline, co-trimoxazole and streptomycin. The range of types of resistance observed was broader among poultry and chicken meat isolates, with notable additional resistance to quinolones and third-generation cephalosporins, which are critically important in human medicine. There was a trend for increased AMR prevalence and MDR among isolates from commercial abattoirs sourcing chickens from medium- and large-scale commercial farms. Tetracycline resistance was most common along meat value chains beginning with small-scale farms, correlating with farmers commonly reporting its use. Increased contamination and differences in AMR patterns were observed between isolates from beef carcasses at the abattoir and those from retail beef in some supply chains, suggesting the possibility of contamination at a later stage during the value chain in that setting. A noteworthy observation from this study was that the AMR patterns of *E. coli* isolated from children under five years of age at outpatient clinics in the meat value chain study areas reflected the commonly used antimicrobials in human medicine, including ampicillin, co-trimoxazole, streptomycin and amoxicillin-clavulanic acid, with lower levels of resistance to third-generation cephalosporins and ciprofloxacin. This suggests that contaminated meat may be just one exposure pathway for humans. The findings emphasize the need for further work to better define such pathways and their relative importance, and the potential for targeted risk management. Nevertheless, food is likely to still be an important vehicle for transmission of resistant bacteria from animals to humans. Additional factors that can drive AMR include environmental contamination with excreted antimicrobials or their metabolites, residue concentrations of antimicrobials in edible tissues, and direct zoonotic transmission (Pruden and Arabi, 2011, Marshall and Levy, 2011, Padungtod *et al.*, 2006, Aarestrup, 2006, O'Neill, 2016).

The loss of effective antimicrobials to treat sick animals adversely affects livestock production and farmers' livelihoods (Cerniglia and Kotarski, 2005). An additional risk for anyone involved in the meat production chain is exposure to resistant bacteria.

For example, farmers working with cattle, pigs and poultry are more likely to be infected with methicillin-resistant *Staphylococcus aureus* (MRSA-398) than other individuals in the community (Garcia-Alvarez *et al.*, 2012, Lewis *et al.*, 2008).

CATEGORIES OF AMU IN ANIMAL PRODUCTION IN RELATION TO THE EMERGENCE OF AMR

Antimicrobial Use

Widespread antimicrobial use is considered to be the main factor associated with resistance in bacterial populations (APUA, 2008, Aarestrup *et al.*, 2008,

Acar and Moulin, 2012). The use of antimicrobials in health care, agriculture, horticulture, aquaculture and industrial settings has an impact on the expression, selection, persistence and transfer of resistance traits in bacterial populations (Aminov and Mackie, 2007, Courvalin, 2008, Mathew *et al.*, 2007, PCIFAP, 2010). Excessive use and misuse of antimicrobials are widely recognized as two of the major drivers for acquired AMR, both directly and indirectly, due to the selection pressure imposed on human and animal microbiota (WHO, 2014a, Novo *et al.*, 2013, PHE, 2014), and on environmental bacteria (Martinez and Baquero, 2009). Many of the antimicrobial substances licensed for veterinary use belong to antimicrobial classes or groups routinely used in humans. Table 3 provides an example of those licensed for use in animals in the EU.

Usage of third-generation cephalosporins (e.g. ceftiofur), deemed as critically important antimicrobials in humans (WHO, 2012), has been associated with the selection of co-resistance to disparate antimicrobials such as tetracycline and chloramphenicol in enteric *Escherichia coli* bacteria (Lowrance *et al.*, 2007). This has been observed in hospitals, farms, wastewater and sewage environments and in the gut of treated animals and humans (Martinez and Baquero, 2009, APUA, 2008). The persistence of antimicrobial residues in feed and animal waste contaminating soil and water also affects the aquatic and environmental microbiomes (You and Silbergeld, 2014). Colistin (polymyxin E) has been used in veterinary medicine for several decades, and is used across different food-producing animal species (e.g. pigs, poultry, sheep, goats, calves and adult cattle) including farmed fish. Indications for usage range from gastrointestinal infections by Gram-negative bacteria to topical treatment of mastitis, colistin is often supplied in feed and water in intensive systems, not only for treatment purposes but also for prophylactic and metaphylactic purposes in groups of animals (Catry *et al.*, 2015, EMA, 2015). Colistin is also currently used in human medicine for the treatment of infections caused by MDR carbapenem-resistant Gram-negative bacteria, in combination with tigecycline, which has led colistin to be reclassified as a highly important antimicrobial by WHO (Catry *et al.*, 2015, EMA, 2015, WHO, 2012). The recent detection of acquired colistin resistance in food-borne pathogens in animals, foods and humans (associated with infection), observed across several countries, raises serious and urgent public health concerns (Figures 2 and 3) (Skov and Monnet, 2016). It is currently recommended that, for veterinary purposes, colistin should only be used for treatment (Catry *et al.*, 2015, EMA, 2015).

However, there are currently limited data on the extent and patterns of antimicrobial usage observed in food-producing animals, particularly in LMICs.

Only a few countries in Europe (e.g. the Netherlands, Denmark, Sweden) currently conduct integrated surveillance of AMU and AMR in humans, animals and food products of animal origin. At European level, the ESVAC (European Surveillance of Veterinary Antimicrobial Consumption) programme assesses antimicrobial sales, adjusted by biomass of livestock populations, across different European countries (ESVAC, 2015). A recent study by Van Boeckel *et al.* (2015) used statistical models based on the data from 32 countries to estimate the extent of antimicrobial usage in food-producing animals at global level (Figure 4).

The emergence of AMR strains is dependent on several factors relating to the antimicrobial itself (e.g. amount, dosage, frequency and duration of selection pressure) and the organism (e.g. presence of genes conferring resistance to that particular substance, and advantage provided by the expression of these to the survival of the bacteria) (McEwen, 2006). Use of antimicrobials may unblock gene expression, resulting in the development of resistance genes in bacteria (Courvalin, 2008, Lambert, 2012) or promoting the occurrence of mutations (Martínez *et al.*, 2007). This kind of selection pressure is an important factor in the dissemination of resistance

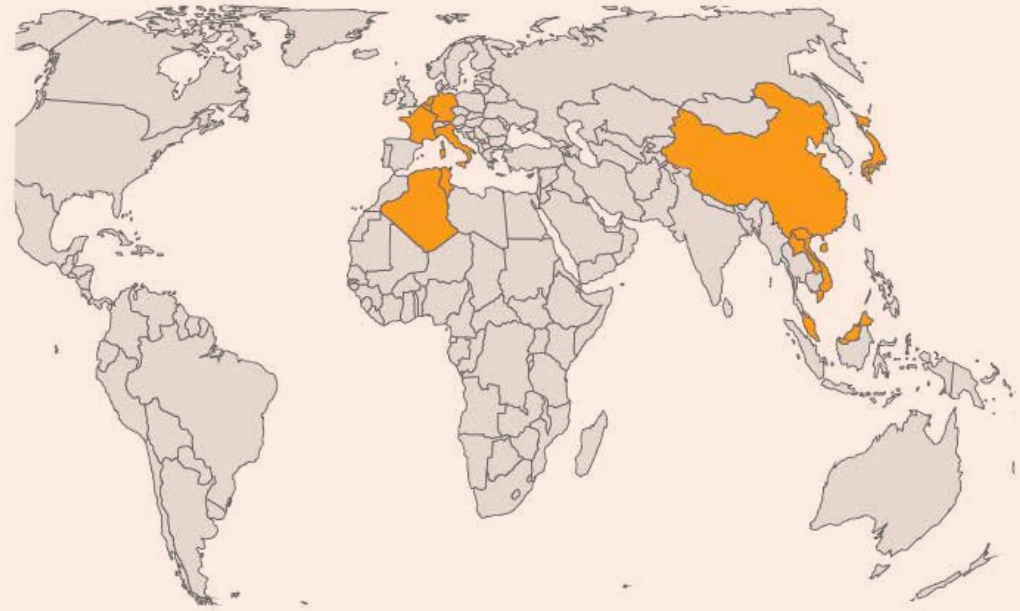
TABLE 3. List of antimicrobial classes licensed for veterinary use in the EU and main indications

Antimicrobial class	Veterinary use in the EU	Major indications	Risk to public health	Hazard of zoonotic relevance	Probability of AMR transfer
Aminoglycosides (e.g. gentamicin, neomycin)	Species: cattle, sheep, goats, horses, dogs and cats	• Septicaemias • Digestive, respiratory and urinary infections	Risk profiling required	Enterobacteriaceae *Enterococcus* spp.	High
Cephalosporins (3rd and 4th generation)	Species: cattle, pigs, horses, dogs and cats	• Septicaemias • Respiratory infections • Mastitis	High	Enterobacteriaceae	High
(Fluoro) quinolones	Species: cattle, pigs, chickens, turkeys, rabbits, dogs and cats	• Septicaemias • Infections (e.g. colibacillosis)	High	*Campylobacter* spp. Enterobacteriaceae	High
Macrolides (including ketolides)	Species: cattle, sheep, pigs, and poultry	• Mycoplasma infections (pigs and poultry) • Haemorrhagic digestive disease and proliferative enteropathies (leitis) associated with *Lawsonia intracellularis* (pigs) • Respiratory infections • (cattle and sheep) • Liver abscesses (cattle)	Low to limited	*Campylobacter* spp. *Salmonella* spp.	High
Penicillins (natural- Lactamase- sensitive)	Species: cattle, sheep, poultry, horses, dogs and cats	• Septicaemias • Respiratory infections • Mastitis	Low or limited	None specific	High
Penicillins (broad spectrum beta-lactamase-sensitive) Aminopenicillins	Species: cattle, sheep, pigs, poultry and dogs	• Pasteurellosis and colibacillosis (poultry) • *Streptococcus suis* infections (pigs) • Respiratory infections (cattle and pigs)	Further risk profiling required	Enterobacteriaceae *Enterococcus* spp.	High
Penicillins (narrow spectrum beta-lactamase resistant)	Species: cattle and sheep	• Metritis • Mastitis	Low or limited	None specific	High
Penicillins (Beta-lactamase protected broad spectrum) - Co-amoxiclav	Species: cattle, pigs, dogs and cats	• Respiratory infections • Mastitis • Metritis • Colibacillosis (cattle and pigs)	Further risk profiling required	Enterobacteriaceae *Enterococcus* spp.	High
Polymyxins (including colistin or polymyxin E)	Species: cattle, sheep, pigs and poultry	• Septicaemias • Colibacillosis • Urinary infections • Gram-negative digestive infections	Currently under evaluation	Enterobacteriaceae	Low*
Rifamycin (rifampicin)	Species: cattle	• Mastitis • Metritis	Low or limited	None specific	High
Tetracyclines	Species: cattle, sheep, goats, pigs, horses and poultry	• Respiratory diseases • Bacterial enteritis • Urinary tract infections • Metritis • Mastitis • Pyodermatitis • Keratoconjutivitis (cattle) • Chlamydiosis • Heartwater • Anaplasmosis • Actinomycosis • Actinobacilosis • Ehrlichiosis • Resistant strains of *Staphylococcus aureus*	Low or limited	*Brucella* spp.	High

*May need to be reassessed in the light of new evidence of the emergence of plasmid-mediated colistin resistance in animals and humans (Catry *et al.*, 2015, Skov *et al.*, 2016).

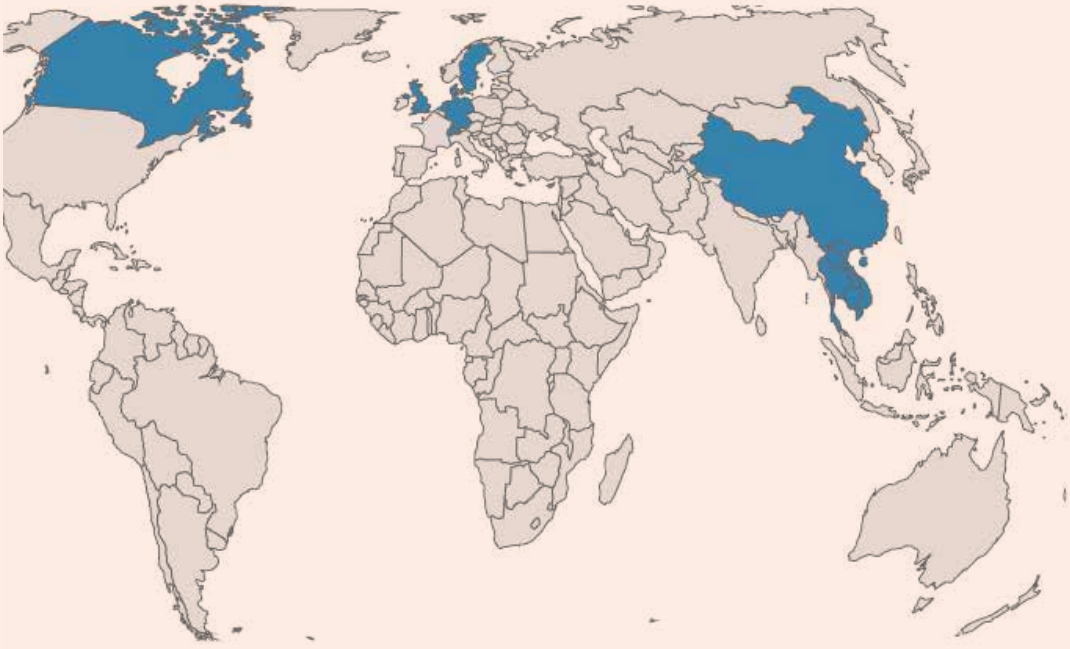
Source: Adapted from EMA, 2014

FIGURE 2. Geographical distribution of detected colistin resistance (mcr-1 gene) in food-producing animals as of March 2016



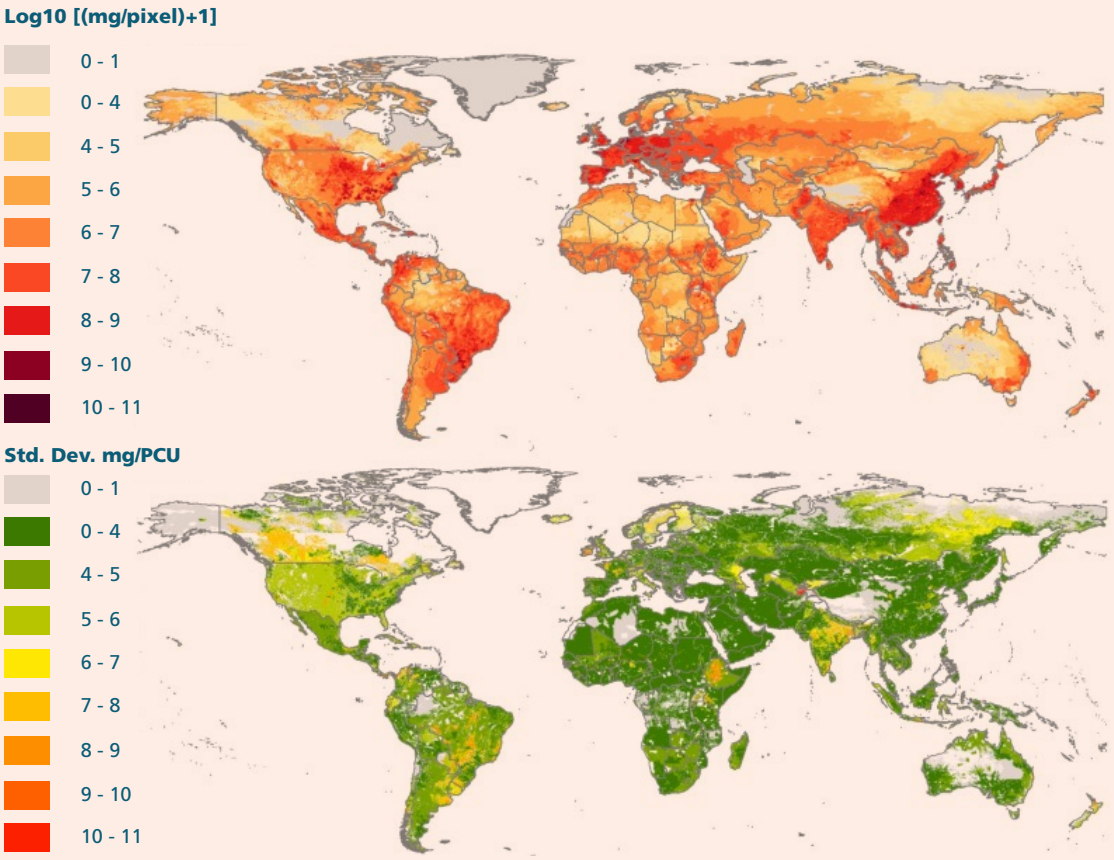
Source: Skov and Monnet, 2016

FIGURE 3. Geographical distribution of detected colistin resistance (mcr-1 gene) in humans as of March 2016



Source: Skov and Monnet, 2016

FIGURE 4. Estimated antimicrobial consumption in food-producing animals at global level in milligrams per 10 km² pixel (top map), and average standard deviation (SD) of estimates of milligrams per PCU (Population Correction Factor), a technical unit of measurement which acts as a proxy for the size of the animal population (bottom map)



Source: Van Boeckel *et al.*, 2015

determinants (Courvalin, 2008). Antimicrobials as disparate as fluoroquinolones and β-lactams may even foster the intra- and inter-cellular mobilization of resistance genes (Amábile-Cuevas, 2012). Prolonged antimicrobial exposure has been associated with acquisition of multidrug resistance in enteric bacteria in both humans and animals (Levy and Marshall, 2004), and also in aquaculture (Nonaka *et al.*, 2007). The use of combinations of antimicrobials may also result in the selection of MDR bacterial strains (Martinez and Baquero, 2009). Use of oral systemic antimicrobials in groups of animals is common practice in conventional farms, particularly in pigs (Burow *et al.*, 2014) and poultry (Apata, 2009).

This places selection pressure on both commensal and pathogenic bacteria in the gut flora, which promotes the exchange of genetic material (Courvalin, 2008, Burow *et al.*, 2014). Antimicrobial usage is particularly high in monogastric species (poultry and pigs), compared to other food-producing animals.

These are typically kept in intensive, indoor production systems at high densities, and are therefore vulnerable to infectious disease challenges (McEwan and Fedorka-Cray, 2002, O'Neill, 2016). In monogastric production systems, the dosage,

frequency and duration of antimicrobial therapy is likely to be high. Since it has been estimated that 75 to 90 percent of antimicrobials used in livestock are excreted, mostly unmetabolized (Marshall and Levy, 2011), the concentration of antimicrobial residues in these farm environments is likely to be high. This is a crucial risk factor for the emergence of AMR.

AMU also impacts on the competition for nutrients between bacterial populations in ecosystems through the elimination of susceptible bacteria (Aarestrup *et al.*, 2008). In a recent simulation model by Volkova *et al.* (2013), plasmid-mediated resistance to ceftiofur in *Escherichia coli* in livestock was affected by the reduction of numbers of susceptible bacteria in the gut microbiota. Use of antimicrobials also appears to reduce the infective dose required by resistant pathogens to cause infection, posing a serious risk for hosts colonized with, or exposed to, these bacteria (da Costa *et al.*, 2013). Although there is evidence of occurrence of resistance in gut commensal bacteria (e.g. *Escherichia coli*, *Enterococcus* spp.) in food-producing animals and foods of animal origin (Chantziaras *et al.*, 2014), there are currently scarce data on the role of these bacteria as potential sources of resistance genes for human and animal pathogens (Courvalin, 2008).

Simulation studies by Volkova *et al.* (2012) have predicted that the survival of resistant commensal bacteria in between antimicrobial doses in livestock can be expected. However, maintenance of resistance would be dependent on bacteria- and plasmid-specific biological and ecological factors, and on the prevalence of resistant bacteria in the host and in the environment (da Costa *et al.*, 2013).

The same issues have been discussed in humans carrying resistant strains and undertaking antimicrobial therapy (da Costa *et al.*, 2013, PHE, 2014). In *Escherichia coli*, transfer of resistance determinants between bacteria has been mainly associated with the selection pressure imposed by AMU (da Costa *et al.*, 2013). Use of β-lactams has been associated with increased levels of MRSA 398 strain isolated in livestock through a co-selection mechanism (ECDC/EFSA/EMA, 2009).

Use of third-generation cephalosporins in livestock has been associated with emergence and spread of ESBLs in Gram-negative bacteria, which poses a serious risk to public health (Aarestrup *et al.*, 2008). The sporadic isolation of carbapenem-resistant Gram-negative bacteria in livestock is also deemed a serious risk to public health, as carbapenems are considered "last-resort" β-lactam antimicrobials for therapy of life-threatening infections in humans. Carbapenems are not currently used in food-producing animals and are predominantly used in human hospital settings (Table 4). Nevertheless, there could be a risk of co-resistance through use of other antimicrobials in agriculture or through horizontal transfer from human pathogens (EFSA, 2013). Transfer of resistance traits within the bacterial cell can be induced by AMU and has been observed with macrolides in Enterobacteriaceae. Erythromycin promotes the transposition of erythromycin-resistant genes from a non-conjugative to a conjugative plasmid, which can then become mobile between bacteria (Courvalin, 2008). In contrast, in countries where use of particular substances (e.g. fluoroquinolones) is discouraged in livestock, low levels of, or no resistance to, these antimicrobials are observed in food-borne zoonotic pathogens (Aarestrup *et al.*, 2008).

Antimicrobials at low dosages (i.e. residual levels, sub-lethal or sub-therapeutic dosages) are also factors contributing to resistance as they promote genetic and phenotypic variability in exposed bacteria (Andersson and Hughes, 2014, You and Silbergeld, 2014, Martinez, 2008), even though they are less likely to kill susceptible bacteria – leading to selection bias – than antimicrobials administered at higher dosages. In addition, sub-lethal dosing also appears to increase gene expression, development of virulence and formation of biofilms that

TABLE 4. Antimicrobial groups currently not licensed for veterinary use in the EU

Antimicrobial class	Hazard of zoonotic relevance	Probability of AMR transfer
Carbapenems and other penems	Enterobacteriaceae	High
Ceftaroline and ceftobiprole (e.g. fosfomycin)	MRSA	Low
Glycopeptides	• *Enterococcus* spp. • MRSA	High
Glycylcyclines	• Enterobacteriaceae • MRSA	Low
Lipopeptides	• *Enterococcus* spp. • MRSA	Low
Monobactams	• Enterobacteriaceae	High
Oxazolidinones	• *Enterococcus* spp. • MRSA	High
Penicillins (carboxypenicillins and ureido-penicillins including beta-lactamase inhibitors combinations)	• Enterobacteriaceae • *Enterococcus* spp.	High
Riminofenazines	None specific	Low
Sulfones	None specific	Low
Drugs used solely to treat tuberculosis or other mycobacterial diseases	None specific	High

Source: adapted from EMA, 2014

are also indirectly responsible for resistance due to the close proximity of bacteria, which may favour the horizontal transfer of mobile resistance determinants (Andersson and Hughes, 2014, Lupo *et al.*, 2012).

Soil (Mathew *et al.*, 2007, Forsberg *et al.*, 2012) and water (Lupo *et al.*, 2012) bacteria have been described as reservoirs for resistance genes, and are exposed to antimicrobial residues derived from human, industrial, and agricultural use (Forsberg *et al.*, 2012) (Figure 5). Persistence of resistant bacteria and resistance genes in the farm environment and in medicated feed has been associated with acquisition of resistance by enterococci bacteria isolated from livestock and poultry and it is currently a major public health issue (da Costa *et al.*, 2013, You and Silbergeld, 2014, IFT, 2006, Acar and Moulin, 2012). Presence of antimicrobial residues derived from anthropogenic, industrial and agricultural usage in the aquatic and terrestrial environments also contribute to selection pressure on environmental bacteria (Lupo *et al.*, 2012, Forsberg *et al.*, 2012, You and Silbergeld, 2014) and commensal and pathogenic bacteria present in the gut microbiota of farmed animals (You and Silbergeld, 2014). It must be noted that antimicrobials differ in how efficiently they are processed in animal guts (and thus in the volume of residue excreted) (Kemper, 2008), and in how long the residues remain bioavailable in the environment (e.g. how quickly they are adsorbed to soil) (Kumar *et al.*, 2005, Kemper, 2008). Therefore different antimicrobials pose different levels of public health risk (AAM, 2009). For example, sulphonamides do not strongly adsorb to soil, thus remaining bioavailable in the environment for relatively long periods (Wegst-Uhrich *et al.*, 2014). Excretion rates are dependent on the type of antimicrobial, mode of administration, animal species and period since administration. Excretion rates for tetracyclines and sulphonamides may vary between 40 and 90 percent, for example (Kemper, 2008). There is currently a lack of data on concentrations of antimicrobials in soil, manure and surface water, perhaps due to insufficiently sensitive analytical methods (Thanner *et al.*, 2016). Importantly, antimicrobials which are concentration-dependent, such as fluoroquinolones and aminoglycosides, are more likely to rapidly exert

selection pressure on bacteria in soil or water before they are diluted, in comparison to time-dependent antimicrobials (such as macrolides and β-lactams) which require sustained high concentrations in order to have an effect on bacterial viability (Amábile-Cuevas, 2016).

The growing prevalence of MDR organisms enables coselection, hence requiring the removal of all antimicrobials in order to achieve a useful reduction in the prevalence of resistance. However, reduction of numbers of resistant bacteria may only be possible if these are outnumbered by susceptible bacteria in an antimicrobial-free environment in which only a small number of individuals have been exposed to antimicrobials, or in the presence of a limited "selection density" (Levy and Marshall, 2004). This will usually not be the case in high-selection-density environments such as hospitals and conventional intensive farms (Levy and Marshall, 2004, PHE, 2014).

Antimicrobial growth promoters (AGPs)

Exposure of bacteria to sub-therapeutic concentrations of antimicrobials is likely to have an important role in AMR evolution (Andersson and Hughes, 2014). The use of AGPs as feed additives in intensively produced animals has been found to alter the gut microbiota of treated animals and promote resistance transfer within the animal and the environmental microbiome (You and Silbergeld, 2014). AGPs are administered at sub-therapeutic dosages to groups of animals via drinking water or feed for prolonged periods to improve growth rates (Wielinga *et al.*, 2014, Capita and Alonso-Calleja, 2013, Castanon, 2007). AGPs are sold and used in many countries without veterinary prescription or supervision (Laxminarayan *et al.*, 2013). There is still conflicting evidence, however, as to whether the improvement in animal production due to the use of AGPs is significant, and the mechanism behind any such effect is still largely unknown (Lee *et al.*, 2012).

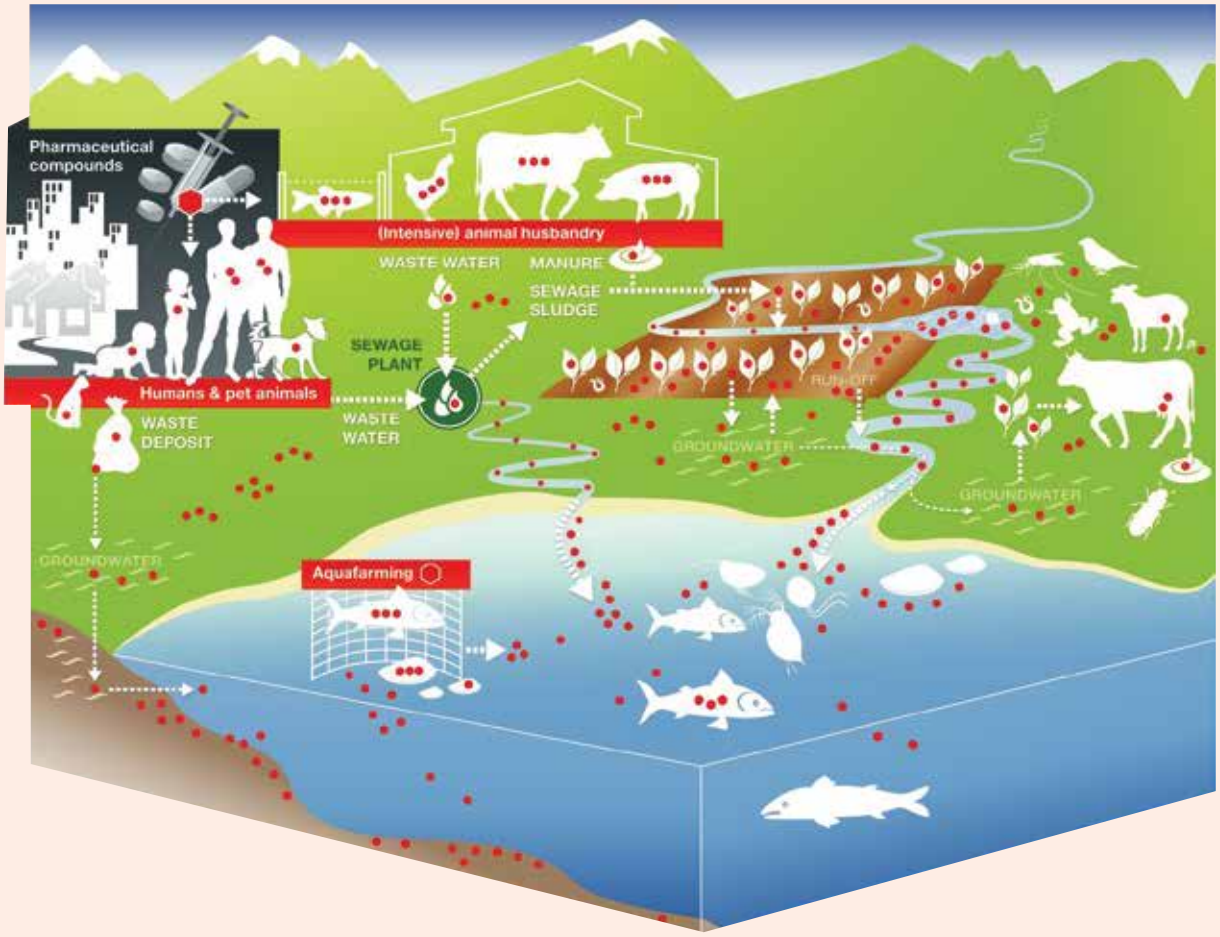
It is important to state that the impact of AGPs on productivity could be as little as 1 percent or lower, if nutrition, hygienic practices and health care of the herd or flock are also improved (Laxminarayan, 2015). However such necessary improvements may not always be easy to achieve, especially in LMICs, where resources are limited. The banning of AGPs in Europe in 2006 (European Commission, 2005) led to a reduction in the levels of vancomycin-resistant enterococci (VRE) previously observed in poultry in Denmark (Singer *et al.*, 2003). Vancomycin was not licensed for use in poultry, but resistance had emerged as result of the use of avoparcin (also a glycopeptide) as an AGP in poultry production (Singer *et al.*, 2003, Wielinga *et al.*, 2014). Even though sub-therapeutic dosages have been linked to the emergence of antimicrobial resistance, AGPs continue to be used in many non-EU countries in intensive animal production, although the extent of this is currently unknown (Singer *et al.*, 2003, Capita and Alonso-Calleja, 2013, Castanon, 2007). There has been a recent move in the United States to reduce their use (BVA, 2012).

Animal feed is supplemented by other, non-antimicrobial compounds, which may, in turn, affect microorganisms. Sepiolite, for instance, has been used as an additive in animal feed since 1990 in the EU. It slows the passage of food through the intestinal tract, enabling a better absorption of nutrients. Sepiolite is not an antimicrobial, nor does it exert any antimicrobial effect, but it does promote the horizontal transfer of resistance plasmids between bacteria, which could be aggravated if there is concomitant presence of AGPs (Rodríguez-Beltrán *et al.*, 2013). This is just one example of the very complex and unpredictable interactions made possible by the use of antimicrobials.

Prophylaxis

This is defined as the administration of an antimicrobial to susceptible but healthy animals to prevent the occurrence of infectious disease. A common example is the infiltration of the mammary

FIGURE 5. Antimicrobial usage in humans, animals and agriculture, and resulting dispersion of antimicrobial residues into aquatic and terrestrial environments (represented by red dots)



Source: Van Boeckel *et al.*, 2014

glands of dairy cattle with antimicrobials such as penicillins, cephalosporins, or other lactams after cessation of lactation (Landers *et al.*, 2012, Capita and Alonso-Calleja, 2013). Such AMU is likely to have a similar effect to that of growth promoters, although therapeutic levels of dosing, if adhered to, should be less likely to induce resistance in exposed bacterial populations.

Nevertheless, this may not be the case when the administration occurs in animal groups through water and feed (e.g. pigs, poultry) due to the variations in consumption by individual animals and the number of animals exposed. It must also be noted that – particularly in countries where antimicrobial production and storage chains are inadequate (due to environmental or infrastructure-related issues) – antimicrobials may be susceptible to degradation through oxidation-reduction reactions, hydrolysis, biodegradation or photodegradation (Osei Sekyere, 2014). These antimicrobial preparations may then have reduced concentration and bactericidal activity when used, allowing for the survival of exposed bacteria and the generation of resistance (Osei Sekyere, 2014).

In addition to the use of antimicrobials in livestock, tetracyclines have been used in honeybee

colonies since the 1950s in the United States to control infections by *Melissococcus pluton* and *Paenibacillus larvae*. The diversity of *tet* genes encoding either efflux pumps or ribosome protection has been detected at high levels in the microbiota of US honeybees, while only exceptionally in bees from countries where tetracyclines are not used (Tian *et al.*, 2012).

Metaphylaxis

Defined as the administration of an antimicrobial at therapeutic doses to all animals within a group in which some individuals have exhibited infection. Metaphylaxis acts both as a treatment for those animals currently infected and a preventive measure against infection in those animals who are healthy but risk becoming infected. The administration of oxytetracycline in the flock water supply, as treatment and prevention against mycoplasma infections in poultry, is a common example. The number of animals exposed to metaphylaxis is often large: in poultry production, medicated water or feed can be used to treat more than 30 000 birds in the same flock. In addition, even if precise dosing is used – for example where antimicrobials are administered to all members of a herd in injectable form – such widespread AMU inevitably increases the risk of resistance emergence, due to the increased probability of bacteria with natural resistance encountering the antimicrobial and potentially being selected for within the affected microbiota.

Therapeutic use

This describes treatment of active bacterial infection in a single animal, or a group, via antimicrobial administration. Whereas even a single dose of antimicrobial administered to a single animal has the propensity to generate AMR within bacterial populations resident in that animal, the repeated and continued usage of antimicrobials, for example to treat recurrent infections, compounds this risk (Usui *et al.*, 2014, Harada and Asai, 2010). Often, broad-spectrum antimicrobials are used in livestock before, or in place of, a confirmed diagnosis (for example before undertaking any antimicrobial susceptibility testing) due to economic considerations. The administration of macrolide antimicrobials such as erythromycin to pigs, regardless of the route of administration, has been shown to select for resistance in *Campylobacter* spp. strains (Harada and Asai, 2010). The duration of systemic treatment should only be long enough to ensure elimination of infection in the affected animal or animal populations as this could result in further selective pressure on the gut microbiota (EMA, 2015). Correct dosing is very important for the reasons stated above. In addition, for antimicrobial substances that have been licensed for veterinary use for many years, recommended dosages by manufacturers in the Summaries of Products Characteristics (SPCs) may not always be adequate as these may have not been calculated in accordance with updated pharmacokinetics and pharmacodynamics principles, or may not have taken account of the evolution of antimicrobial susceptibility in bacterial populations (EMA, 2015).

It is important to note that when antimicrobials are administered via largely unregulated vehicles such as feed or water – whether for therapeutic, metaphylactic or prophylactic purposes – the exact intake of individual animals will be hard to ensure and define, and sub-optimal dosing may occur (particularly of sick animals within a group housing and/or an *ad lib* feed and water system), increasing the risk of AMR emergence.

Biocide use

These are substances which, through chemical or biological action, hinder the activity of a broad spectrum of microorganisms (SCENIHR, 2009, IFT, 2006).

Not only are they commonly used in agricultural settings – their use is also frequent in human health-care systems and at community level. They may lead to emergence of AMR through

cross-resistance, co-resistance and clonal drift mechanisms, and by activating an SOS response in bacteria leading to the repair and integration of DNA, some of which may include resistance genes (Capita and Alonso-Calleja, 2013, Davin-Regli and Pagès, 2012). Biocide use within the agricultural industry can be divided into two broad categories: a) animal feed preservatives and b) disinfectants and antiseptics. Within the food-production industry, biocides may also be used as food preservatives or decontaminants. Examples include sulphites, lactic acid, trisodium phosphate or acidified sodium chlorate. Such compounds inhibit the growth of microorganisms in, or on, foodstuffs and produce (Capita and Alonso-Calleja, 2013).

Lower susceptibility and resistance to biocides has been reported in bacterial populations since the 1950s (Davin-Regli and Pagès, 2012). Plasmids, transposons and integrons often also carry genes conferring resistance to biocides (e.g. disinfectants and antiseptics) and to heavy metals, providing an evolutionary advantage to the resistant bacteria even in the absence of antimicrobial pressure (Martinez and Baquero, 2009, Acar and Moulin, 2012). Resistance mechanisms are similar for biocides and antimicrobial substances: selection pressure from biocide use – in food production, industrial, agricultural and human health care settings, and water and wastewater treatment facilities – could result in cross- or co-selection for AMR (Davin-Regli and Pagès, 2012, SCENIHR, 2009). Biocides and antimicrobial substances may share common target sites (SCENIHR, 2009) and can be located closely together in mobile units (e.g. plasmids), leading to co-resistance (Levy and Marshall, 2004). Efflux pumps coded at chromosomal level have been involved in resistance to both antimicrobials and biocides (e.g. quaternary ammonium) due to their non-specific mechanism (Cambau and Guillard, 2012). Resistance to biocides has been associated with stress responses in bacteria, particularly when in the presence of sub-lethal doses but also in the presence of other stressors in the environment (e.g. osmotic and oxidative pressure, pH, nutrient availability) (IFT, 2006). Non-compliance with recommended dilution, preparation and storage of biocides may explain the increased tolerance to these products at low or sub-lethal concentrations and changes in phenotypic expression (e.g. membrane permeability, changes in membrane charge, efflux pumps, biofilm formation) of exposed bacterial populations (Davin-Regli and Pagès, 2012, SCENIHR, 2009). There is currently a paucity of data relating to the extent of biocide use, presence of environmental residues and environmental stability (SCENIHR, 2009). Although risk assessment for AMR occurrence due to exposure to biocides is now a mandatory requirement for registration and licensing of these substances in European countries (Anon., 2012), there is still little information on the correlation between biocides and antimicrobial resistance (Oggioni *et al.*, 2015).

Quaternary ammonium compounds or ethanol, for example, are used to destroy or inhibit microorganisms in animal husbandry and food production and processing facilities. In a recent study, *Listeria monocytogenes* and *Salmonella enterica* strains exhibited reduced susceptibility to chlorine dioxide and peroxyacids when exposed to increasing concentrations of these chemicals over time. In addition, the resistance of these bacterial species to various antimicrobials also increased after disinfectant exposure. Prior exposure to acidic disinfectants also increased the percentage of *L. monocytogenes* bacteria surviving subsequent acid treatments (SCENIHR, 2009).

Despite several existing studies providing evidence of a role of biocides in the emergence of AMR, exceptions exist where only weak or moderate correlations were observed between phenotypic biocide resistance and AMR in some bacteria such

as *Staphylococcus aureus* (Oggioni *et al.*, 2015). Therefore, further research is needed to assess the impact of biocides on pathogens relevant to public health. Since such substances are used ubiquitously and in large quantities throughout the food chain, it may be surmised that their relative impact on AMR emergence within agriculture and food industries may be important. Nevertheless, in order to quantify further the repercussions of biocide use on the emergence of AMR within and outside the agricultural industry, further in-field surveillance of biocide use, and research into potential causal associations, is warranted (Fraise, 2002). It must also be noted that the use of biocides is very widespread in many industries, and the disease burden to humans and domestic animals without their use would need to be weighed against any potential benefits from their reduced use.

Animal feed preservatives

Preservatives such as citric acid or sodium benzoate protect animal feed against decay caused by microorganisms. Such organic acids when ingested by food-producing animals may induce a selection pressure on gut bacteria (SCENIHR, 2009). In addition, these preservatives are often added in large quantities to feed such as silage, an increasing trend globally. This silage, if stored in such a manner that effluent can contaminate the environment, may potentially extend selection pressure to environmental bacteria.

Heavy Metals

Heavy metals may be used in agriculture as part of livestock feed supplements, and in a Chinese study were detected in manure from pig farms (Zhu *et al.*, 2013). Heavy metals have been associated with the emergence and spread of AMR in environmental bacteria due to co-selection.

The presence of heavy metals has also been associated with the reduction of susceptibility of bacterial populations in soil (Aminov and Mackie, 2007) and commensal bacteria (e.g. enterococci) (Werner *et al.*, 2013) to antimicrobials. Heavy metals in soil could be derived from mining and industrial activities but also from agriculture and health care (e.g. mercury in dental amalgams) (Aminov and Mackie, 2007). AGPs used in livestock production can also contain heavy metals as trace elements (e.g. copper, zinc), or medication (e.g. arsenic in coccidiostatics) (You and Silbergeld, 2014). These metals can co-select for AMR not only in the gut microbiota but also in the environment through their persistence in animal waste (You and Silbergeld, 2014). Commensals and pathogens in the gut microbiota of animals could also be exposed to heavy metals through contaminated feed (e.g. mercury in fishmeal) (Defra, 2014, You and Silbergeld, 2014).

Other potential sources of resistance emergence and maintenance

One hypothesis is that stress and resistance genes in the bacterial genome are located closely together, which would promote their co-expression under stressful conditions, even in the absence of AMU (Mathew *et al.*, 2007). Stressors identified as associated with emergence and transfer of resistance include extreme temperatures and variations on osmotic pressure and pH that could have an impact on the integrity of the DNA and affect bacterial survival (Aarestrup *et al.*, 2008). Lack of biodiversity in ecosystems – often due to human action – also seems to drive the emergence of resistance determinants and bacteria (da Costa *et al.*, 2013).

Transfer of resistant bacterial clones to hosts (i.e. humans and animals) is dependent on the age and health status of the host, and the frequency of contacts between the host and the environment, and/or between humans and animals (Martinez and Baquero, 2009, Mathew *et al.*, 2007). Host stressors such as weaning have been described as influencing the prevalence of AMR as they might have an impact on the gut environment, either by enhancing uptake

of resistance genes by bacteria or by favouring the survival of resistant strains (Mathew *et al.*, 2007). Finally, a number of stress conditions in urban areas, especially those in LMICs, have been related to the selection or maintenance of AMR genes in potentially pathogenic bacteria (Rosas *et al.*, 2011). Conditions as apparently unrelated to antimicrobials as air pollution might foster the resistance of airborne bacteria to antimicrobials (Jiménez-Arribas *et al.*, 2001).

Risks of agricultural antimicrobial usage, other than AMR selection

Antimicrobial usage of any kind implies a risk for AMR selection and spread. We currently lack adequate risk-assessment models for exploring the impact of agricultural AMU, simply because we have a poor understanding of the complex processes that lead to the emergence and spread of AMR. Many such mechanisms, e.g. mutations and horizontal transfer between distantly-related bacteria, occur at very low rates, often below our detection capabilities. However, as bacterial populations are enormous and many of them still unknown (we have been able to culture less than 10 percent of the species of the human microbiota, and less than 1 percent of the soil microbiota), most of these very rare phenomena at individual organism level must be occurring frequently at population level. In addition, there are other unpredictable implications of AMU in livestock. Statutorily acceptable levels of oxytetracycline and erythromycin in meat, following use in food-producing animals, can disrupt the fermentation process of sausages, as they are able to inhibit microbial starter cultures, but may allow the growth of pathogens such as *S. typhimurium* and *Escherichia coli* O157:H7 (Kjeldgaard *et al.*, 2012).

This shows that the use of antimicrobials in food-producing animals may lead to food-related outbreaks through unexpected pathways.

AMR EMERGENCE AND AMU WITHIN DIFFERENT ANIMAL PRODUCTION SYSTEMS

Aquaculture

Antimicrobials are widely used in aquaculture for therapeutic, metaphylactic and prophylactic purposes. AMR in aquaculture can occur through direct exposure to antimicrobials delivered as group therapy to fish, or through livestock and human effluents containing resistant bacteria, resistance genes and antimicrobial residues. These will then exert selection pressure on gut microbiota of fish and on other bacteria in the aquatic environment (FAO/OIE/WHO, 2006). The extent and persistence of antimicrobial residues in these production systems is currently unknown, but they are likely to be greatly diluted in the environment.

The presence of antimicrobial residues in sea sediment could be due to constant exposure of fish to medicated feed and antimicrobial group treatments (Muziasari *et al.*, 2014). It is worth noting that no antimicrobial agents have ever been developed solely for fish or shellfish therapy, in part due to the difficult and expensive registration process for antimicrobial drugs (Scarano *et al.*, 2014, Rodgers and Furones, 2009). Therefore, the substances widely used in aquaculture are the same as those licensed for therapy and prophylaxis of infectious diseases in humans and livestock.

These include commonly used drugs (Capita and Alonso-Calleja, 2013, FAO/OIE/WHO, 2006), including substances currently deemed as critically important in human medicine (Table 5).

Resistance determinants to commonly used antimicrobials such as fluoroquinolones, tetracyclines and florfenicol have been detected in aquatic bacteria, some of which are also human pathogens such as *Escherichia coli* (Miranda *et al.*, 2013). Resistance genes and bacteria resistant to sulphonamides and trimethoprim have also been isolated from the sediment under aquaculture farms in the Baltic Sea and

TABLE 5. Antimicrobial agents and classes used in aquaculture and their importance in human medicine

Antimicrobial group	Antimicrobial substance	Route of administration	Importance to human medicine
Aminopenicillins	Amoxicillin	Oral	Critically important
	Ampicillin	Oral	Critically important
Amphenicols	Chloramphenicol**	Oral/Bath/Injection	Highly important
	Florfenicol*	Oral	Highly Important
Macrolides	Erythromycin	Oral/Bath/Injection	Critically important
Aminoglycosides	Streptomycin	Bath	Critically important
	Neomycin	Bath	Critically important
Nitrofurans	Furazolidone	Oral/Bath	Important
	Nitrofurantoin**	Oral	Important
Fluoroquinolones	Oxolinic acid	Oral	Critically important
	Enrofloxacin*	Oral/Bath	Critically important
	Flumequine	Oral	Critically important
Tetracyclines	Oxytetracycline	Oral/Bath/Injection	Highly important
	Chlortetracycline	Oral/Bath/Injection	Highly important
	Tetracycline	Oral/Bath/Injection	Highly important
Sulphonamides	Sulphonamides	Oral	Highly important

* Licensed only for veterinary use.
** Banned for use in commercial aquaculture in most fish-exporting countries since 2002.
Critically Important for human medicine are those antimicrobials which meet both Criterion 1 and Criterion 2 (see below).
Highly Important are those antimicrobials which meet either Criterion 1 or Criterion 2.
Important are those antimicrobials which meet neither Criterion 1 nor Criterion 2.
Criterion 1. An antimicrobial agent that is the sole, or one of limited available therapies, to treat serious human disease.
Criterion 2. An antimicrobial agent used to treat diseases caused by either: a) organisms that may be transmitted to humans from non human sources or, b) human diseases causes by organisms that may acquire resistance genes from non human sources (WHO 2012).

persisted in the environment for at least 6 years, however, there was no evidence of spread to nearby aquatic areas (Muziasari *et al.*, 2014).

Nonetheless, these kinds of sediments could act as reservoirs of resistance genes and bacteria in local fish farms and in humans via food distribution (FAO/OIE/WHO, 2006). Most marine bacteria cannot yet be cultured (Suzuki *et al.*, 2013), therefore it is possible, although speculative, that there may be as yet undetected reservoirs of resistance in the aquatic environment.

Land-based intensive systems

The intensification of livestock production (i.e. large numbers of animals kept at high density and usually indoors) has been associated with the use of antimicrobials as prophylaxis against infectious disease, often for prolonged periods and for large populations of animals. Pro- and metaphylactic use of antimicrobials at different stages of livestock production can also have an impact on the emergence of resistance (Salyers, 2001). In Poland, higher levels of resistance have been reported in *E. coli* isolates in piglets. This contrasted with the predominant *E. coli* isolates with susceptible pheno- and genotypes reported in sows in the same study (Mazurek *et al.*, 2013). It was associated with the prophylactic use of antimicrobials in younger animals to prevent and contain the spread of respiratory and gastrointestinal infectious diseases (Mazurek *et al.*, 2013).

Animals bred for intensive production also tend to have reduced variability in their microbiota and a similar susceptibility to colonization with particular bacterial species (Schokker *et al.*, 2014). This, coupled with the close proximity of animals

in such systems, could result in amplification of any resistant population(s) of bacteria, which may outcompete other bacterial populations. Again, partially due to the factors above, an intensive system run with poor biosecurity and herd/flock health may run a high risk of being colonized by pathogenic strains of bacteria (Zhu *et al.*, 2013).

Should poor animal health within such situations also necessitate the increased use of antimicrobials, this is likely to support the development of AMR (FAO, 2013b). Given ever-growing global demand for livestock products, it is expected that intensive production will continue to expand in the future. It may be hypothesized, however, that intensive systems with high biosecurity may, in fact, reduce requirements for AMU and thus reduce the risk of AMR emergence. Intensive farms may also be able to take practical steps to mitigate AMR transfer into and out of the system. But as the authors could find little evidence to substantiate these theories in the literature, further research is warranted.

It is important to note that, while hypotheses can be made about the effect of agricultural practices on the emergence of AMR in food animals, biological factors need to be considered in relation to the potential for transmission of resistance to human bacterial populations. A systematic review by Lazarus *et al.* (2015) found that poultry appeared to be a more likely source for a proportion of human ESCREC infections than other food-producing animals (Lazarus *et al.*, 2015).

Genomic data "have demonstrated that human extraintestinal pathogenic *E. coli* and avian pathogenic *E. coli* share numerous virulence factors" (Johnson *et al.*, 2007), and "resistant strains that are able to infect avian sources are also more likely to possess the cellular machinery required to infect humans" (Lazarus *et al.*, 2015). Such findings are relevant from a public health perspective since the fractional proportion of poultry products consumed globally currently outstretches any other protein source, and is projected to continue to do so (due both to increasing global demand and the efficiency of poultry feed conversion, which surpasses that of other livestock) (FAO, 2013a).

Land-based extensive systems

Extensive livestock farming systems, typically characterized by low inputs generating low outputs (the converse of intensive systems) may potentially require lower inputs of antimicrobials, and thus by default, result in lower rates of AMR emergence.

However, by comparison with intensive systems, extensive systems require higher animal numbers for the same output. The Indian smallholder dairy subsector is one example (FAO, 2013b). Extensive systems involving free-roaming animals in large numbers may exhibit high commensal and pathogenic bacterial transmission rates and exposure to multiple bacterial species (including environmental species such as soil bacteria) which may not be as prevalent in intensive systems (FAO, 2013b). These factors may result in promoting the generation and transmission of AMR genetic material and bacterial populations.

Organic systems

Organic production systems in different countries can vary in the level of antimicrobial therapies allowed. In Europe, restrictions exist in the number of therapeutic courses allowed and the duration of withdrawal periods (Anon., 2007). Pro- and metaphylactic use of antimicrobials is prohibited. Alternative therapeutic plans are encouraged, and use of antimicrobials is only permitted when necessary.

Use of vaccines for disease prevention is permitted and encouraged (Anon., 2007, Mazurek *et al.*, 2013). Recent studies comparing AMR levels in livestock reared in organic versus conventional production systems showed higher concentrations in the latter (Mazurek *et al.*, 2013, Cui *et al.*, 2005,

Holtcamp, 2011). In Poland, Mazurek *et al.* (2013) reported that resistant *E. coli* isolates were mainly observed in cows raised in barns in conventional farms rather than in cows having access to pasture and raised organically, with lower exposure to antimicrobials (Mazurek *et al.*, 2013). In another study in the United States, MDR *Campylobacter* spp. strains were detected in both antimicrobial-free and conventional pig farms. This is likely due to environmental reservoirs that could be a source of resistance genes and resistant bacteria (Quintana-Hayashi and Thakur, 2012).

The authors do suggest, however, that in a poorly managed organic system, the drive to reduce AMU may lead to the administration of doses of antimicrobials below the minimum inhibitory concentration (MIC), leading to an increased selection pressure for AMR bacteria and/or recurrent infections or extensive onward transmission, requiring repeat treatment of single or multiple animals and instigating selection pressure for AMR.

In addition, in organic systems where livestock production is integrated with an extensive and/or a free-range or outdoor farming model, access to AMR genes or bacterial populations via soil bacteria and effluent may result in a propensity for organic/extensively-produced livestock to harbour AMR comparable with conventionally produced or indoor animals. One study conducted on pigs, which was the first to document the isolation of ciprofloxacin-resistant *Campylobacter coli* in the United States, is a case in point (Gebreyes *et al.*, 2005). More comparative research is required on this topic, though it may be suggested that high biosecurity, high herd/flock health and indoor, organic systems may potentially induce and harbour relatively less AMR than others. Despite this, it should be noted that biocide treatment of organically-produced animal feed and human foods may still potentially induce AMR in the food chain. The indiscriminate use of biocides should therefore be discouraged (Davin-Regli and Pagès, 2012, Fernández Fuentes *et al.*, 2014).

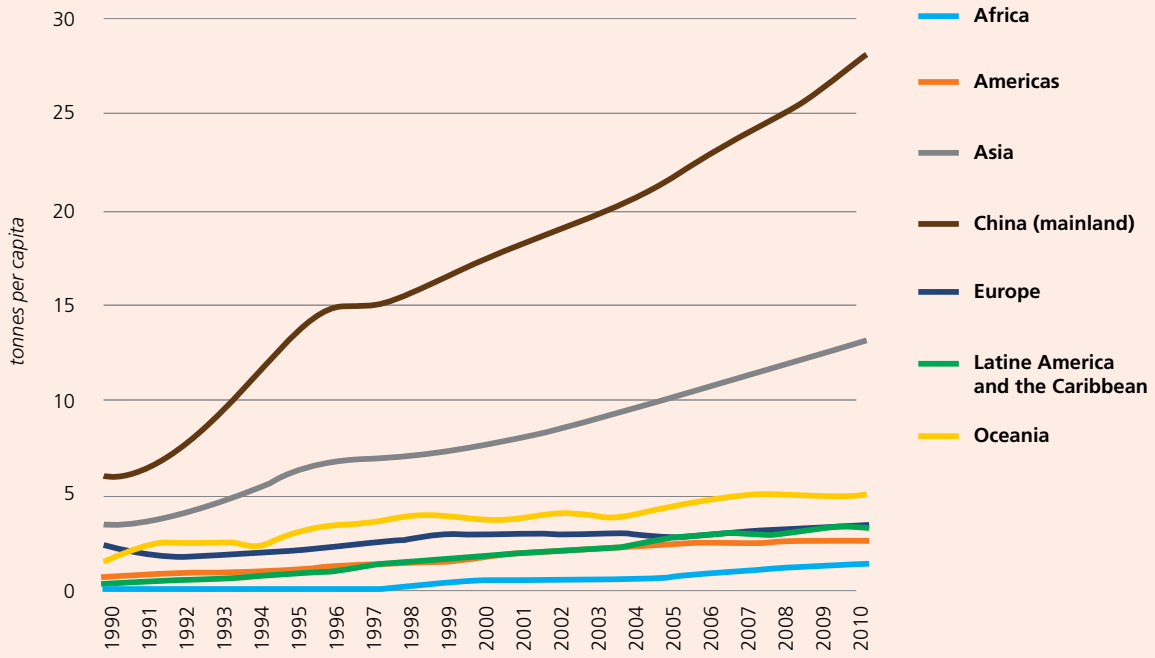
Risk factors for the emergence of AMR in agriculture at national and international level

It is important to highlight the fact that the extent and patterns of AMU in agriculture and other industries are likely to vary considerably between and within countries, due to the influence of various factors. These may include, but are not limited to:

- legislative framework and governance;
- financial status and stability;
- degree of international imports and exports;
- human resources: population size, education and expertise;
- culture;
- structure and organization of the various agricultural production systems in use nationally.

In many countries, particularly LMICs, there have been dramatic changes in agricultural systems in recent years, driven by both increasing local demand and new and emerging trade opportunities (HBF, 2014, Rushton, 2010, FAO, 2013a, FAO, 2013b, Otte *et al.*, 2007). A growing global population and increasing wealth in emerging economies, for example in China and India (O'Neill, 2001), has stimulated demand for animal protein and the development of global value chains (Otte *et al.*, 2007). For example, new export opportunities for sub-Saharan Africa (USDA, 2014) have led to increased production and intensification of agricultural systems in the region, with most recent figures showing 2.5 percent annual growth in total cereal production over the last decade, total meat production doubling in the last 20 years, and egg and milk production also expanding at rapid and sustained annual rates (FAOSTAT, 2014). Globally, poultry production has been growing this century at around 3 percent per year and seems set to continue to grow as global diets and consumption patterns shift (FAO, 2013a). Fish production by aquaculture has been rising over the last 20 years, from 13 percent of total world fish supply

FIGURE 6. Aquaculture fish production by region



Source: Adapted from FAO, 2013a

in 1990 to 40 percent in 2010 (FAO, 2013a). The growth of aquaculture has been most significant in Asia, especially China, and also in Latin America and Africa as shown in Figure 6. Aquaculture is a fast-expanding agricultural sector in many LMICs, and the unregulated use of antimicrobials in many of these countries poses serious risks of AMR developing and spreading at local and global level – the latter through international trade (Heuer *et al.*, 2009). However, the negative impact of detection of residues in seafood in international trade has led to improved practices and certification of aquaculture by national regulatory bodies and international certifying agencies.

Changes to agricultural systems as a result of intensification involve changes in livestock/fish numbers, feed type and quantity used, husbandry methods, and animal density. All of these factors can influence disease dynamics (Otte *et al.*, 2007), which in turn may drive changes in AMU. The extent of the impact on AMU depends on the attitude of veterinary practitioners and farmers towards use of antimicrobials within particular legislative and governance frameworks, and alternative methods for maximizing animal productivity.

MECHANISMS OF SPREAD OF ANTIMICROBIAL RESISTANCE BETWEEN ANIMALS AND HUMANS

LOCAL AND GLOBAL MECHANISMS OF SPREAD

Both pathogenic and non-pathogenic resistant bacteria can be transmitted from livestock to humans via food consumption, or via direct contact with animals or their waste in the environment (Marshall and Levy, 2011). Fomites can also play an important role in the local and wider spread of resistant bacteria. In Denmark, farm-to-farm spread of multidrug-resistant *Salmonella enterica serovar typhimurium* DT204 has been closely studied, and shared farm equipment (e.g. machinery) was identified as an important route (Aarestrup, 2006).

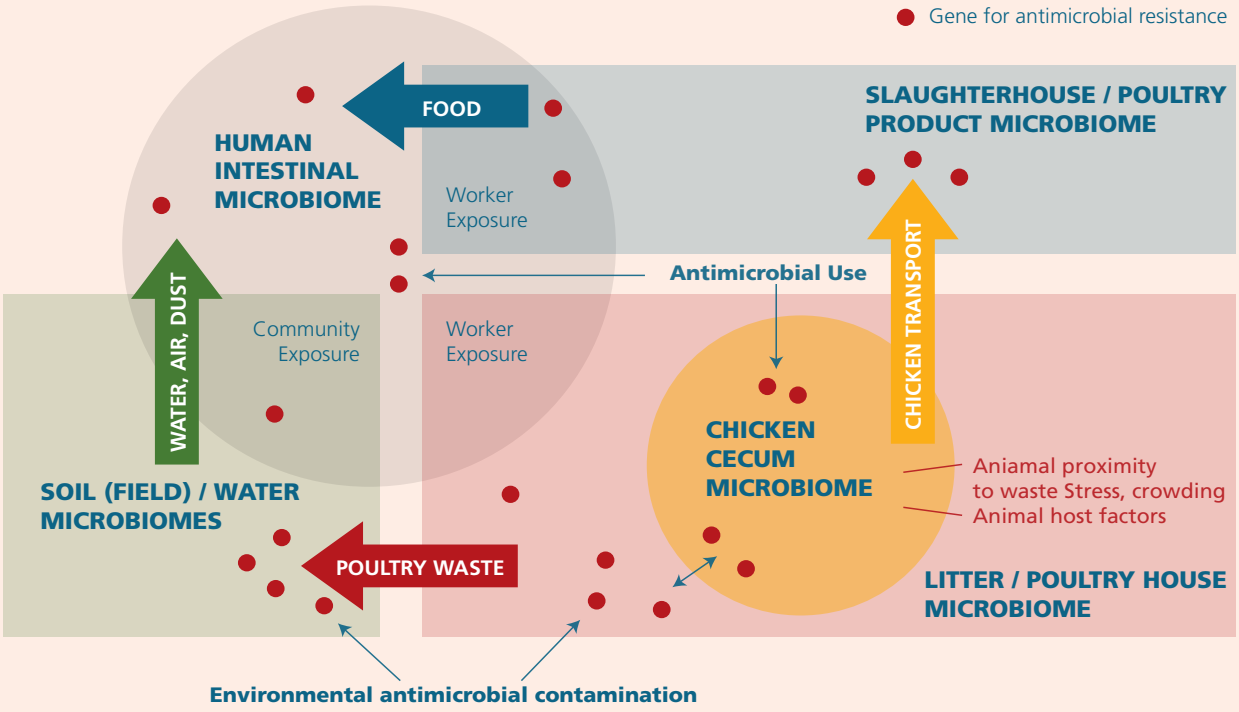
Any mechanism that helps spread bacteria has the potential to transfer resistant bacteria. Resistance may also be conferred by the exchange of genetic elements between bacteria of the same or different strains or species, and such transfer can occur in any environment where resistant bacteria have the opportunity to mix with a susceptible bacterial population, such as in the human or animal gut, in slurry spread on agricultural soil, or in aquatic environments (Woolridge, 2012, Aarestrup, 2006, Baquero *et al.*, 2008). An example of a framework in which resistance genes could spread from poultry production to humans is shown in Figure 7. If resistance develops in environmental bacteria, this can create an animal or human health problem when such bacteria contaminate water, food crops or animal feed, introducing the opportunity for bacterial mixing with commensal or pathogenic species in the animal or human gut (Aarestrup, 2006, Finley *et al.*, 2013, Marti *et al.*, 2013).

Risk pathways for the spread of AMR via the environment

Many antimicrobial preparations used for livestock are given orally so that antimicrobial residues excreted in animal faeces have the potential to exert selection pressure on bacterial populations in soil or water (Woolridge, 2012, AAM, 2009), as shown in Figure 5. However, evidence is scarce as to how important this mechanism is in transferring resistance (Hong *et al*., 2011, McEwen, 2006, Novo *et al*., 2013, Woolhouse *et al*. 2015), and different antimicrobials have different fates in the environment (Kumar *et al*., 2005, Kemper, 2008, AAM, 2009). It must be considered that residues resulting from human treatment with antimicrobials or from pharmaceutical manufacturing can also exert selection pressure on environmental bacteria (Igbinosa *et al*., 2011, Baquero *et al*., 2008, Finley *et al*., 2013, Wellington *et al*., 2013, Novo *et al*., 2013). Indeed, effluent from drug manufacturing has been found to contain extremely high concentrations of antimicrobial residues, as previously reported in countries with large pharmaceutical industries such as India (Larsson *et al*., 2007, Sim *et al*., 2011, Mutiyar and Mittal, 2014, O'Neill, 2015).

Water, including that treated for human consumption, is an important vehicle for the spread of AMR. Water is not only directly consumed by humans and animals, but is used for irrigation of crops which are then consumed by humans or used as animal feed (Finley *et al*., 2013) (Figure 5). Water can spread antimicrobial residues, resistant bacteria and resistance genes far and wide through the flow of natural water bodies and anthropogenic influenc-

FIGURE 7. Conceptual framework for the spread of AMR genes in a poultry production system



Source: Davis *et al.*, 2011

es such as irrigation. This is a significant concern in LMICs, where water has been shown to be a major route for transmission of pathogenic bacteria to humans (Wellington *et al.*, 2013). Recreational water use has also been linked to exposure to AMR bacteria (Leonard *et al.*, 2015).

It has recently been found that antimicrobial residues, AMR genes and bacteria can spread for some distance via airborne particulate matter from large cattle feedlots in semi-arid areas of the United States (McEachran *et al.*, 2015). These areas are prone to soil scouring, dust formation and strong winds. Communities living nearby are therefore exposed to antimicrobial residues, AMR bacteria and genes via direct inhalation of contaminated dust or deposition of particulate matter onto skin, food or water (McEachran *et al.*, 2015).

In different environments, the relative importance of livestock sources of antimicrobial residues and AMR genes versus human sources will vary and the overall contribution of livestock waste to such environmental transmission pathways still remains unclear (AAM, 2009, Wellington *et al.*, 2013, Marti *et al.*, 2013). There are considerable gaps in current knowledge, in part because environmental sites such as flowing watercourses are difficult to study due to their dynamic nature and of water's diluting effect (Woolridge, 2012). Human sources of contamination in the environment make it difficult to ascertain the contribution of livestock production to the environmental spread of AMR. While several studies from various regions have linked the presence of resistance in the environment with contamination by waste from livestock or aquaculture

(effluent, wastewater, or manure), such transmission pathways are necessarily inferred rather than proven (Woolridge, 2012, Binh *et al*., 2007, Acar and Moulin, 2006, Zhao *et al*., 2010, Hong *et al*., 2011, Heuer *et al*., 2002, Heuer and Smalla, 2007, Quintana-Hayashi and Thakur, 2012, Li *et al*., 2012).

According to a recent review by Luby *et al*. (2016), the vast majority of environmental bacteria cannot be cultured using current methods. However, novel molecular techniques may be able to help fill these knowledge gaps. Metagenomics (the study of genetic material recovered from microbial communities) and whole genome sequencing (WGS) are emerging techniques which can provide more detailed characterization of environmental microbiomes and therefore greater insight into the role of the environment as a reservoir of AMR (Penders *et al.*, 2013, Schmieder and Edwards, 2012). AMR genes and other targets of interest such as pathogenicity islands or transposons can be identified from sequencing results, and quantified using publicly available online databases such as the Comprehensive Antibiotic Resistance Database project (CARD) (McArthur *et al.*, 2013), although databases are still not yet well-populated (Luby *et al.,* 2016). One advantage is that the presence of AMR genes can be viewed within the broader context of the whole environmental microbiome – for example analysis of HGT markers can provide information on how AMR may have spread into an environment (Luby *et al.* 2016). Metagenomics has also been used to assess the efficiency of sewage treatment in removing AMR genes (Yang *et al.*, 2014). Several high-income countries are currently adopting and developing metagenomics and WGS techniques to support their surveillance efforts, particularly in the monitoring and detection of bacterial strains relevant to public health, and the carriage and diversity of resistance genes within these. WGS allows the characterization of the full resistance genotype while WGS applied to RNA allows investigation of expression of resistance genes in bacterial isolates (Chan, 2016). There is still room for improvement of the methodologies, as well as a need for better bioinformatics to combine and analyse the sequence data (Clausen *et al.,* 2016). Molecular techniques are becoming more affordable for routine use in high-income countries, but the cost is still prohibitive for most LMICs.

Risk pathways for the spread of AMR via food distribution

As a consequence of the inherent challenges associated with data collection on environmental spread, food-borne transmission often becomes the primary focus for studies of livestock-to-human spread of AMR (Woolridge, 2012). Meat contamination is undoubtedly easier to study, so there is some bias in favour of researching this transmission route. As a result, there is a considerable body of evidence describing the food distribution network as a risk pathway for transmission and spread from animals to humans. In Kenya, *E. coli* isolates from retail beef samples were found to be resistant to ampicillin (31 percent), tetracycline (20 percent) and nalidixic acid and ceftazidime (4 percent), with 27 percent showing multidrug resistance (Kariuki *et al.*, 2013). In the United States, the latest report on contamination of retail meats highlights cephalosporin resistance levels being above 2002 levels, while AMR levels in *Salmonella* from retail chicken were reported at 20 percent (a reduction from 38 percent in 2009 and 28 percent in 2012) (NARMS, 2013). MDR *Salmonella* was detected in all retail meat sources, although the proportion of MDR isolates declined between 2011 and 2013. Ceftriaxone resistance in *E. coli* isolates from retail chicken increased from 8 percent in 2002 to 13 percent in 2011.

Any food contaminated with resistant bacteria provides a direct route for human colonization (Hong *et al.*, 2011, Marti *et al.*, 2013). Capita and Alonso-Calleja (2013) assert that, in quantitative terms, transmission of AMR via food is likely to be the most important known pathway from livestock

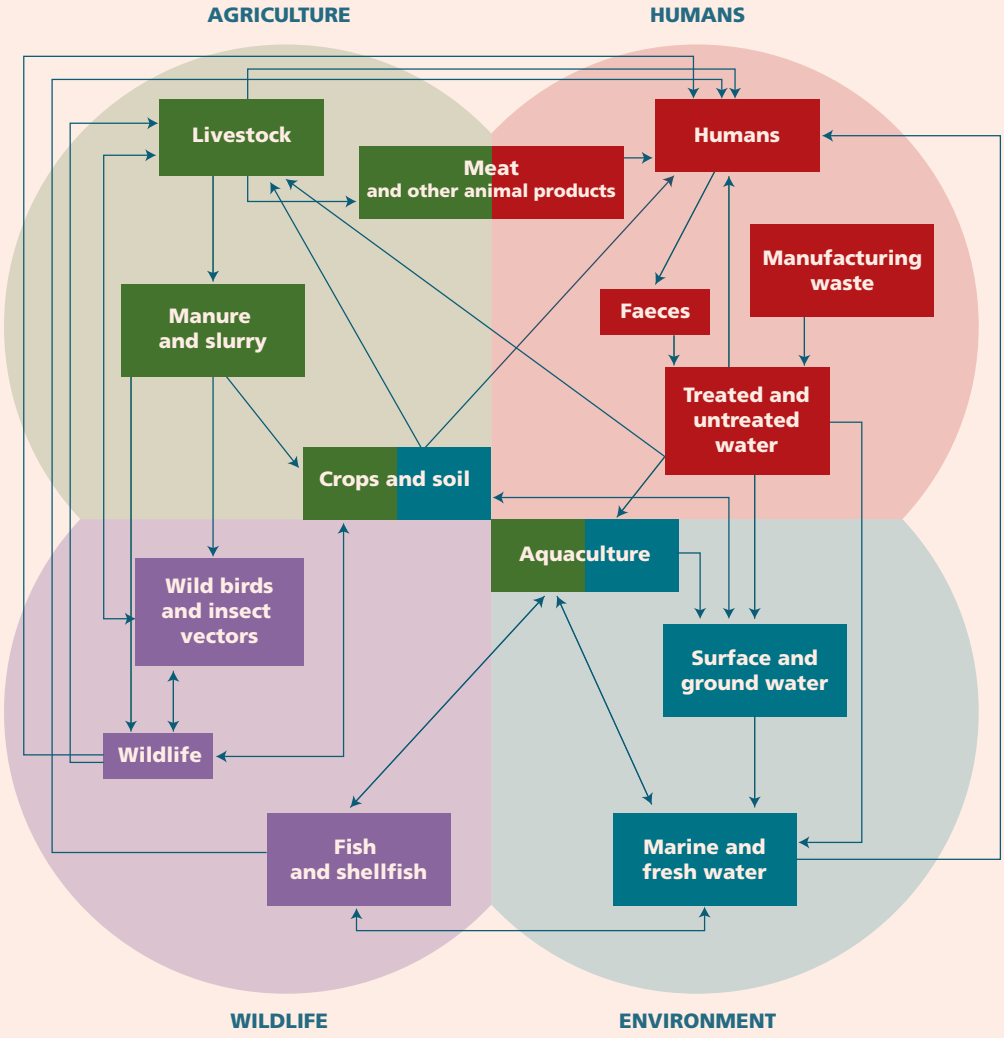
to humans (although it should be recognised that transmission via the environment – to which humans are continually exposed – is still poorly understood and has not yet been quantified). Trade in food products and human travel could have significant roles in the spread of AMR, both locally and globally. In a globalized world, people and products are transported around the earth in a matter of days, and AMR bacteria and resistance genes are disseminated with them. This has been demonstrated in a number of studies of imported meat, fish and dairy products (Ozawa *et al.*, 2002, Skov *et al.*, 2007, Wilson, 2003, Noor Uddin *et al.*, 2013, Zhao *et al.*, 2003, Warren *et al.*, 2008, Hong *et al.*, 2011). Higher incomes in the emerging economies, the changing demands of consumers, and improvements in transportation technology mean that perishable foods, including animal-derived products, are now more easily shipped around the world than ever before (Aarestrup, 2006). As a consequence, strains of resistant bacteria can quickly reach areas where they had previously been uncommon or unknown (Okeke *et al.*, 2005). The global trade in food products is expected to keep increasing in future, both in terms of volume and geographical coverage.

Despite convincing evidence for the existence of potentially important risk pathways for food-borne transmission of AMR, direct evidence for AMR in humans resulting from consumption of food products is very limited. This may be in part because hygiene procedures during meat processing can be very effective at removing bacteria. In a study of cattle from three beef feedlots in Nebraska, Schmidt *et al.* (2015) detected third-generation cephalosporin and trimethoprim-sulfamethoxazole-resistant *E. coli* on 100 percent of hides but only 0.5 percent of carcasses and 0 percent of retail meat from the same animals. According to Wielinga *et al.* (2013), concern about the use of avoparcin as an AGP arose in Denmark during the 1990s due to some evidence of a link to vancomycin resistance in humans. Use of vancomycin in European hospitals was low, but levels of resistance in humans were high. Studies involving vegetarians and non-vegetarians showed a prevalence of about 20 percent vancomycin-resistant enterococci (VRE) among meat eaters while none were detected in vegetarians. There are still considerable knowledge gaps around the risk of AMR emergence in humans associated with the consumption of animal-derived foods, and further research is urgently required.

The effect of low concentrations of antimicrobial residues on the human gut microbiome has also still to be elucidated. The Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) recommends Maximum Residue Limits (MRLs) for veterinary drugs in foods. The Codex MRL is the maximum concentration of residue recommended as legally permissible or recognized as acceptable in or on a food. It is based on "the type and amount of residue considered to be without any toxicological hazard for human health as expressed by the Acceptable Daily Intake (ADI) […] It also takes into account other relevant public health risks as well as food technological aspects […] Furthermore, the MRL may be reduced to be consistent with good practices in the use of veterinary drugs and to the extent that practical analytical methods are available" (Codex Alimentarius, 2015a).

A number of studies in Africa have reported unacceptably high antimicrobial residues in poultry meats (Dipeolu and Alonge 2002, Muriuki *et al.*, 2001). A cross-sectional study of retail pork in suburban and urban districts in Hanoi, Vietnam, found that 5.5 percent of all meat samples from retail shops contained tetracycline residues (Duong *et al.*, 2006). In China, 7.7 percent of aquatic food products were found with levels of residues that were unacceptable for human consumption (Hao *et al.*, 2015). Recently, emerging evidence suggests that even very low concentrations of antimicrobial residues in foods could potentially alter the AMR characteristics of human intestinal bacteria. This

FIGURE 8. Potential transmission pathways of antimicrobial-resistant bacteria, resistance genes and antimicrobial residues, at the agriculture-human-environment-wildlife interface



Source: Adapted from Wellington *et al.*, 2013

is, however, a complex and novel field of research, and there are still few studies addressing this important issue (Cerniglia *et al.*, 2016). The amount of residues available to bacteria in the human gut is greatly affected by dose, the extent of binding to gut contents, and metabolism (Cerniglia *et al.*, 2016). It should be noted that while there are international guidelines for MRLs in food (WHO, 2008, Codex Alimentarius, 2015b), there are no water quality guidelines regarding the presence of resistant bacteria or antimicrobial residues in fresh water used for human and animal consumption or crop irrigation.

The application of manure to crops intended for human consumption is a possible pathway for the spread of AMR from animals to humans (Kumar *et al.*, 2015, Tang *et al.*, 2015). However, Marti *et al.* (2013) found that resistant bacteria on vegetables

grown for human consumption were ubiquitous regardless of farming system or geographical location, thought to be due to the naturally-occurring and ancient presence of AMR in soil bacteria. Furthermore, manuring the soil did not increase the prevalence of resistant bacteria on vegetables sold for consumption. Higher levels were detected when vegetables were harvested from soil where manure was still present, indicating that the period between application and harvesting could be optimized and is a critical control point.

Diverse opportunities for spread

Pathways of environmental and food-borne spread of AMR are complex and varied. Figure 8 illustrates the diversity of interactions at the interface between agriculture, humans, the environment and wildlife through which the spread of AMR bacteria, resistance genes, or antimicrobial residues can potentially occur. The relative importance of each pathway in terms of risk is not demonstrated in Figure 8 due to the fact that many of these are still ambiguous, based on current knowledge.

The vast majority of AMR spread is not monitored or studied and thus the importance of transmission pathways and the magnitude of spread is largely unknown. For example, resistance to synthetic and semi-synthetic antimicrobials has been recently detected in faecal samples from monkeys, tapirs and felids in wild habitats of southern Mexico, the source(s) and transmission pathways of which are as yet unknown (Cristobal-Azkarate *et al.*, 2014). As 70 percent of emerging zoonotic diseases originate in wildlife (Jones *et al.*, 2008), the presence in wildlife of resistance to critically important antimicrobials is a significant public health concern.

Clearly, there are diverse opportunities for environmental spread of AMR as well as the perhaps more straightforward risk pathways of food distribution. It should also be noted that some of the pathways shown in Figure 8 can operate at global as well as local level, and that many pathways operate in multiple directions. For example, there are pathways from humans to food animals as well as vice versa, creating complex feedback loops.

RELEVANCE AND INFLUENCE OF ANIMAL PRODUCTION ON THE SPREAD OF ANTIMICROBIAL RESISTANCE

International trade

Recent changes in the global patterns of trade in agricultural products have influenced the patterns of spread of bacteria and therefore the spread of AMR around the world (Finley *et al.*, 2013, Aarestrup, 2006). Major exporting countries are at risk of increased resistance acquisition due to the pressure to intensify agriculture to produce greater yields for export. On the other hand, international trade demands could potentially foster more prudent use of antimicrobials in exporting countries. For example, good aquaculture practice certifications have been put in place in several LMICs as a result of import rejections in international markets due to detection of antimicrobial residues (FAO, 2012, FAO, 2011). Emerging economies rapidly opening up significant new markets become substantial importers of agricultural products and potentially import new bacteria with resistance genes selected for by the antimicrobials used in the country of export.

Global trade patterns are shifting due to increased demand and investments in agriculture. Africa as a whole is a net importer of meat and dairy products, while Latin America is a significant exporter of meat products, mostly from Brazil. Asia overall still imports considerably more meat and milk than it exports, however China is a now major exporter of meat products and fish while also importing a considerable amount of meat, fish and dairy products. Thailand and India are major exporters, while Vietnam is a significant importer of meat products. Both

Thailand and Vietnam export a considerable amount of fish. Europe as a whole is a major importer and exporter of livestock products, while Australia and New Zealand are net exporters of meat, fish and dairy products (FAO, 2013a, FAO, 2014). The emerging economies of Brazil, Russia, India, China and South Africa (BRICS) are likely to contribute to changing global trade patterns. In emerging economies, there is likely to be an increase in AMU to secure productivity and ensure animal health in order to keep pace with the rapid expansion into new global markets. There is thus an opportunity for increasing spread of AMR around the world in terms of both prevalence of AMR and diversity of resistance genes.

Aside from the potential global spread of AMR via trade in food products, live animals are also traded on an international scale for breeding and slaughter purposes, and this may also represent an important transmission pathway. While the numbers of animals moved around the globe may not compare to the vast scale of the trade in food products, live animals are carrying large amounts of bacteria in their intestinal tract, which are continually mixing, exchanging genetic material and being excreted. In comparison with the amount of bacteria found on the surface of traded meat products, this animal microbiome represents a considerably higher contamination risk. Breeding animals are usually introduced to importing countries at the top of a breeding pyramid and thus have the potential to spread AMR to a large number of other farms (Aarestrup, 2006). While breeding animals are generally subjected to more rigorous health checks than live animals traded for slaughter, it is not known whether such checks routinely include testing for AMR.

Animal feed is also traded around the globe, and *Salmonella* spp. has often been detected in imported feed, especially where it is produced in LMICs or contains animal proteins (Hsieh *et al.*, 2014, Aarestrup, 2006). A significant amount of water from shrimp farms is shipped along with shrimp in frozen blocks, transported from farms directly to international consumers. This water can contain antimicrobial residues and AMR bacteria (Carvalho *et al.*, 2013, Reboucas *et al.*, 2011, Zhang *et al.*, 2011, Holmström *et al.*, 2003, Le and Munekage, 2004), which may then come into contact with kitchen surfaces, other foodstuffs, and consumers themselves, enabling the global spread of bacteria and resistance genes.

It is important to note that the legislative environment in each country – along with the strength of institutions to achieve widespread compliance – will influence the risk of emergence and spread of AMR within exporting countries and thereby influence the risk of international spread (FAO, 2014).

Influence of different types of agricultural systems on AMR spread

The type of agricultural system and basic infrastructure and services also influences the risk of animal-human-environmental transmission of pathogens at a more localized level, and these factors vary widely from country to country (FAO, 2013b).

Intensive production systems will mean frequent, localized contact between livestock and humans involved with production or living in the area (Rushton, 2010) – and increased risk for transfer of AMR genes and resistant bacteria between animals, humans and the environment (Van Boeckel *et al.*, 2015, Wardyn *et al.*, 2015). Intensive livestock systems produce large quantities of waste, much of which is disposed of on nearby land, potentially increasing the risk of transfer of AMR genes to bacteria in the environment and to pathogenic or commensal bacteria in wildlife (Otte *et al.*, 2007, Hong *et al.*, 2011). Many large-scale intensive systems import animal feed and breeding stock on a global scale (Rushton, 2010), potentially introducing novel strains of pathogenic and non-pathogenic resistant bacteria, which can then mix with the existing microbial communities in the intensive farm. Animal waste from these systems can also spread these resistant bacteria and resistance genes locally to farm workers, who then take them

into their community (Wardyn *et al.*, 2015), and via manure and slurry spreading to the surrounding environment. In low-income settings, many workers on intensive farms are also smallholder farmers themselves, while poultry from intensive systems are frequently sold at live bird markets (Rushton, 2010). This opens up opportunities for spread of AMR from intensive systems into local communities. In the future, if production systems across the world continue to move towards intensification, the environmental spread of AMR may become a more important route than is currently perceived (Aarestrup, 2006). Animal products from intensive farms, and local crops grown using their manure, are likely to be destined for export or wide domestic distribution.

Smallholder systems also have inherent risk pathways for the spread of AMR, but of a different nature. AMU by smallholders is likely to be less than in larger, more intensive farms, but it is also likely to be less controlled. Drugs may not be quality-assured, dosages may not be optimized and in some systems antimicrobials are more likely to be used without veterinary supervision (Katakweba *et al.*, 2012). In intensive farming, inputs and outputs are closely managed to maximize gain and little is avoidably wasted. The cost of antimicrobial treatment is offset against gains in animal health and the growth rate of animals. In smallholder systems, antimicrobials may be used less efficiently (Suriyasathaporn *et al.*, 2012), which may result in increased excretion of antimicrobial residues into the environment. Sub-optimal doses or inappropriate drugs can drive selection for resistant bacteria within the animal gut, leading to the increased presence of resistant bacteria in livestock waste. In smallholder systems, animals are commonly in frequent and close contact with humans and wildlife, and often have freedom to roam and graze among animals from neighbouring farms or among human dwellings. AMR can therefore spread between farms and from livestock to humans. Wildlife grazing on shared land may be at increased risk of colonization with resistant bacteria via animal faeces. If resistant bacteria are transferred to humans within these systems, they can spread readily and become endemic within the local community. The food distribution risk from smallholder systems in LMICs is considerable as animals may be slaughtered and butchered outside of abattoirs and without any formalized food-safety controls. However, meat and other animal products are typically consumed locally, often by the owners of the livestock themselves, so that the spread of AMR may remain limited to local communities. Some smallholders may sell produce at local markets, and this provides potential for more widespread dispersal. Inadequacies in biosecurity controls at live animal markets in LMICs can mean that bacteria, and therefore AMR, are transferred easily between animals and humans within the market environment (Cardona *et al.*, 2009). Compared with intensive systems, the global biomass of animals raised in smallholder systems is likely to be far less and therefore the volumes of antimicrobial residues and resistant bacteria excreted are also likely to be lower, meaning that there is arguably less transmission into the environment overall from these systems. However, smallholder systems are numerous and ubiquitous in all countries and most ecological zones, and therefore provide the potential for resistant bacteria to spread into diverse environments. In agro-ecological systems, where the environment may historically have had low exposure to AMR, the potential transfer of AMR into the environment is of particular concern.

Medium-sized farms present a different set of risk pathways. They are typically small businesses or family-run operations, and contribute considerably to global food security. AMU is likely to be less closely monitored and regulated than in intensive systems, and antimicrobials may therefore be used in a less controlled way. Biosecurity controls, especially in pig and poultry farms, are likely to be much less strictly applied than in intensive systems. Although the volume of waste produced from these systems is much lower than from intensive systems,

waste disposal is likely to be less tightly regulated so that manure and slurry may be disposed of inappropriately (such as in nearby watercourses), or inadequately treated before disposal. Medium-sized farms are more likely than smallholder farms to sell animal products across large distances, perhaps through a marketing and distribution network, and may export some of their produce internationally. The intrinsic risk of AMR emergence in these systems, coupled with the potential for wide distribution, means that these systems are of concern in terms of their ability to spread AMR.

In LMICs, there are likely to be few or no waste-treatment facilities. Adelowo *et al.* (2014) explain that farming systems in Nigeria often dispose of waste by dumping, meaning that the local environment and freshwater supply can become contaminated with resistant bacteria and antimicrobial residues. Similarly, contamination of the environment with human sewage can also be a major factor in spreading AMR bacteria and genes to both humans and animals. The pathways of AMR transfer in such systems are likely to be complex and multi-directional, involving feedback loops between humans and animals.

Aquaculture in LMICs often features an integrated system which uses domestic farm and poultry waste as fish feed (Suzuki and Hoa, 2012). Antimicrobial residues or resistant bacteria from animal husbandry are therefore continuously introduced into aquatic systems and may contribute to a reservoir of resistance genes in fish farms, as has been found in Tanzania and Pakistan (Shah *et al.*, 2012). Farmed fish are an important global food source, and in China, the world's largest producer of farmed fish, the industrialization of production is leading to increasing use of antimicrobials. Furthermore, resistance to the older antimicrobials such as tetracycline has led to increasing usage of quinolones, which are critical in human medicine. High levels of plasmid-mediated quinolone resistance (PMQR) genes have been found in aquaculture systems in China, higher than in swine, poultry or human isolates (Jiang *et al.*, 2012). The selection pressure of large quantities of antimicrobial residues in water can lead to the spread of resistance among aquatic bacteria. Novo *et al.* (2013) found that tetracycline residues in urban wastewater were significantly associated with higher prevalence of resistance, although not just resistance to tetracycline. This highlights the complexity and gaps in our knowledge concerning the mechanisms of transmission within aquatic environments. Muziasari *et al.* (2014) found few AMR genes in the environmental sediments below Baltic Sea fish farms, where there was no influence from human or agricultural systems, concluding that selection pressure in this environment was minimal. The impact of aquaculture practices on the spread of resistance in aquatic systems remains largely unknown, however, and evidence-gathering is complicated by a variety of factors. For example, studies in both China and Egypt have also found ESBL genes in aquaculture systems which are attributed to pollution of these systems with human sewage (Jiang *et al.*, 2012, Ishida *et al.*, 2010).

We need to know much more about the impact, in different types of agricultural production systems, of the use of antimicrobials on the spread of AMR into the environment (Rushton *et al.*, 2014), and in particular we lack data from LMICs (Adelowo *et al.*, 2014).

FURTHER SPREAD OF ANTIMICROBIAL RESISTANCE: CAN IT BE STOPPED?

The consequences of the recent dramatic global changes in food consumption, international trade, agricultural production systems, and human travel in terms of AMR spread and circulation are as yet scarcely known. The recent emergence and spread of colistin resistance in animals, food and humans at international level reflects this (Figures

2 and 3). If the selection pressure imposed by the use of antimicrobials was completely removed this would not necessarily stop the circulation of AMR. This is an "easy to get and hard to lose" problem according to Salyers *et al.* (1997), because resistance is very difficult to reverse due to the ability of genetic transfer elements to adapt to new hosts and new environments (Aminov and Mackie, 2007). Österblad *et al.* (2001) explain that restrictions on AMU are still extremely useful, however, because the prevalence of resistance found in wild animals is still low in areas where the use of antimicrobials in agriculture has historically been low. This indicates that the release of AMR genes into the environment may be an important point for intervention in controlling the spread of AMR (Aminov and Mackie, 2007).

Antimicrobial residues in the environment are not monitored in the same way as are other hazardous substances, e.g. the Dangerous Substances Directive 2006/11/EC (European Commission, 2006) in the European Union. Thus their concentration in the environment is likely to be underestimated or unrecognized. Antimicrobials are often large and complex molecules which biodegrade and behave differently to the archetype chemicals typically used in predictive models of environmental fate (Berkner *et al.*, 2014). Some antimicrobials are not readily biodegradable and may persist at high concentrations for long periods so that future development of more biodegradable antimicrobials might help to reduce the risk of environmental spread and circulation (Wellington *et al.*, 2013).

There is also a need for novel strategies of water quality improvement (Lupo *et al.*, 2012). A considerable amount of research has been conducted into the improvement of waste water treatment due to concerns over pharmaceuticals with undesirable effects on wildlife, such as contraceptives or painkillers. Improving the ability of waste water treatment plants to remove these pollutants would also help to lower environmental concentrations of antimicrobials with similar molecular size, particularly in aquatic environments. Switzerland is one country that has already adopted such strategies (Berkner *et al.*, 2014).

While the environmental proliferation of AMR is becoming increasingly recognized as an important control point, efforts to mitigate AMR spread have largely focused on food distribution until now, due to the more significant knowledge base regarding risk pathways and the fact that controls are easier to implement in food distribution systems. Strategies that minimize the risk of hazardous food-borne bacteria spreading, such as hygiene measures during slaughter and meat processing, and following "Hazard Analysis and Critical Control Point" (HACCP) protocols, help to reduce such risk (Schmidt *et al.*, 2015, WHO, 2014b). Hsieh *et al.* (2014) showed that the presence of *Salmonella* spp. in animal feed could be reduced by monitoring and identifying critical control points at the stages in processing where control could be most effectively applied, recommending standardized control procedures for animal feed processors. Unfortunately, the present situation in LMICs of high AMU combined with inadequate resources and infrastructure to ensure rigorous hygiene during slaughter and meat processing does present significant challenges. It would also be advantageous to reduce or cease completely the transportation of live animals for breeding purposes, as it is possible instead to trade in embryos or semen, thereby avoiding the transportation of large numbers of bacteria in animal guts (Aarestrup, 2006).

Given our current limited knowledge of transmission pathways, options to mitigate the global spread of AMR involve controlling its emergence in various environments, and minimizing the opportunities for AMR to spread along what may be the most important routes. There are clearly numerous opportunities for AMR to spread at local and global scale, and there are still large knowledge gaps as to

what the most important routes are.

Mitigation strategies are indeed possible, and require a joint approach based on agricultural, medical and environmental interests. (Wellington *et al.*, 2013). The WHO draft Global Action Plan (GAP) for AMR draws attention to the use of good sanitation, hygiene and other infection prevention methods to curb the initial emergence and spread of AMR, these being important in mitigating the circulation of AMR in agriculture, humans and the environment (WHO, 2014b). The Codex Alimentarius Code of Practice to Minimize and Contain Antimicrobial Resistance provides guidance on the responsible and prudent use of antimicrobials in food animals. The FAO Technical Guidelines for aquaculture certification (FAO, 2011) provide a framework for countries to implement regulated and responsible use of antimicrobials in aquaculture.

Because livestock, humans and the environment are intimately connected, it is important to consider the emergence and spread of AMR from a "One Health" perspective, which provides a framework for an interdisciplinary approach to dealing with this enormous challenge (Finley *et al.*, 2013, Robinson *et al.*, 2016).

RECOMMENDATIONS

Finally, we present a set of specific recommendations to address the knowledge gaps highlighted in this technical paper:

- The extent of transfer of resistance genes between bacteria in the gut environment of humans and animals should be investigated to quantify the impact of AMU on bacterial populations.
- The dynamics and interactions of genes and microbes within microbiota, microbiomes and different scales of microbial ecosystems, and the transfer of resistance within those, need to be better understood. This will require use of data generated by molecular techniques such as metagenomics together with epidemiological data in an integrated analysis. Databases of molecular sequences are expected to improve over time as usage increases, and bioinformatics techniques need to be developed in order to keep pace with the data challenges associated with the outputs of emerging new sequencing techniques.
- Antimicrobial resistance genes and ICEs can be horizontally transferred between different microbial ecosystems. Being able to better predict the emergence and spread of resistant bacterial clones in the environment and human-agriculture interfaces will help to inform risk-assessment and management strategies. Molecular sequencing and epidemiological studies of resistant bacteria and resistance determinants are required to support risk assessment and simulation (modelling) studies.
- Standardized approaches should be used to create databases of resistance genes and mobile elements encoding resistance traits, and information should be shared freely, such as via the EU project COMPARE (COMPARE, 2015).
- Pharmacodynamic and pharmacokinetics studies are required to assess how antimicrobials interact with microbial populations, particularly in the context of treatment of infections, in order to improve the efficacy of therapy and minimize the risk of AMR emergence. Studies involving sampling prior, during and post systemic antimicrobial exposure of livestock and aquatic animals are needed. This also should be applied to humans and to environments where contact between environmental, commensal and pathogenic bacteria is likely to occur (e.g. sewage).
- Changes in the human intestinal microbiome as a result of ingestion of low levels of antimicrobial residues in food can be studied using metagenomic and analytical chemistry approaches, in combination with bioinformatics. This will enable improved risk assessment for maximum residue limits in foods.
- The association between AMU on farms and AMR among food-borne bacteria, as well as the relationship between AMR in livestock and the incidence of resistant infections in humans, need to be quantified as a priority. This should include the direction and extent of transfer of resistance determinants and resistant strains occurring between and among animals, humans and the environment. There is an urgent need for improved data collection in this regard, especially from LMICS. In order to improve data collection there is a need for robust infrastructure and capacity – currently lacking in many countries – to monitor and investigate AMU and AMR.
- Surveillance for AMR should include more emphasis on epigenetics (using molecular techniques and bioinformatics) to allow tracing the origin of emergence. Integrated surveillance should be conducted for AMU and AMR in food-producing animals and humans. Furthermore,

AMR should also be assessed in food of both animal and non-animal origin. Wildlife species should be investigated as sentinels in surveillance programmes for resistance determinants and resistant bacteria in the environment. Further studies are needed to collect data on the extent and diversity of the resistance gene pool present in the environment. Priority should be given to the development of lab capacity and the training of veterinary and lab staff in LMICs to carry out novel molecular sequencing techniques, in order to support the establishment of AMR surveillance programmes in LMICs.

- Selection pressure is observed even when antimicrobials are used responsibly, in compliance with current recommendations and guidelines. It is therefore important that the risk of AMR is assessed in the context of all antimicrobial usage practices, including usage that is compliant with legislation and recommendations.
- Antimicrobial residues in the environment should be monitored regularly in the same way as other hazardous substances.
- Water treatment is an important control point for selection pressure and human/animal exposure. This aspect should be included in all strategies to reduce AMR risk.
- Development of highly biodegradable antimicrobials should be prioritized in order to reduce the pressure of environmental contamination with antimicrobial residues.
- The use and misuse of biocides should be considered when assessing AMR risk. Further research is needed to assess the impact of biocides and heavy metals on AMR emergence in bacteria. Further in-field surveillance of biocide and heavy metal use, and research into potential causal relationships, is warranted.
- Intensive livestock production methods should be improved by identifying the most efficient systems with regards to minimizing environmental contamination with antimicrobial residues and resistant pathogens, taking into account local conditions and needs, and ensuring sustainability. The benefits of better feed, water, biosecurity and management standards need to be assessed.
- Epidemiologically and cost-effective hygiene practices must be applied within all farming systems and food sectors wherever possible to reduce human exposure to (resistant) pathogens. Use of HACCP protocols is strongly recommended in environments where food is processed and handled.
- A "One Health" approach is essential to improve the efficiency of AMR research, surveillance, prevention and control systems. Harmonized responses and guidelines for AMU and AMR emergence investigation/tracing should be formulated with the integration of animal and human health systems and institutions. Using a "One Health" approach requires a deeper, interdisciplinary understanding of food systems, the drivers of human behaviour within these systems, and the factors which influence how society uses livestock.

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APPENDIX 1

REVIEW PROTOCOL

Study Question: Drivers, dynamics and epidemiology of antimicrobial resistance in animal production: a critical review of the relationship between antimicrobial use in animal production (including aquaculture) and AMR emergence and spread in animals and humans.

Review of the scope and effect of the issue of AMR in agriculture (PIO):

- Population = humans, livestock and fish or other aquatic species connected to animal production and food distribution.
- Issue = relationship between animal production practices and AMR emergence and spread and vice versa.
- Outcome = qualitative and quantitative categorization of the role of animal production practices in AMR emergence and spread.

Search Strategy and Sources

As time and resources did not permit a systematic review of primary literature, the search strategy involved identifying relevant reviews, reports and secondary literature via recommendations from global experts in the field of AMR. The list of references in gathered articles then lead to identification of further sources in specific areas where there were gaps (following initial review). It was recognized as important to include as broad a range of literature as possible and experts from a wide variety of institutions and countries in order to minimize the risk of bias in the gathering of literature.

Sources included:

- review articles (secondary literature);
- government, NGO and Private Agency Reports;
- raw data;
- grey literature;
- expert opinion, see Potential Collaborators Identified, below.

Experts were contacted from:

- Australian National University, Australia;
- University of Guelph, Canada;
- European Medicines Agency, Belgium;
- Danish Institute for Food and Veterinary Research, Denmark;
- Technical University of Denmark, Denmark;
- University of Copenhagen, Denmark;
- Friedrich Loeffler Institute (FLI), Germany;
- Utrecht University, the Netherlands;
- Kenya Medical Research Institute (KEMRI), Kenya;
- Lusara Foundation, Mexico;
- Swedish National Veterinary Institute (SVA) Sweden;
- SAFOSO AG, Switzerland;
- Royal Veterinary College, UK;
- Animal and Plant Health Agency, UK;
- Veterinary Medicines Directorate, UK;
- U.S. Department of Agriculture, USA.

Management of Search Results

All gathered literature was stored in EndNote reference management software.

Inclusion/Exclusion Criteria

- Is the paper relevant to animal production (defined as the rearing of animals including aquatic species)?
- Does the paper relate to the study question?
- Is the article published or does it relate to work carried out within the last 15 years?
- Is the full text available for review?
- Is the paper available in English?

Screening of Search Results

Based on the inclusion/exclusion criteria, one reviewer reviewed the title and abstract (or equivalent) of each article.

Data Extraction

A data extraction form was drawn up using specific headings corresponding to the population, issue and outcome (PIO), based on the study question. This facilitates continuity and reliability of data extraction between researchers.

One reviewer extracted data from each article. Data were stored in a Microsoft Excel spreadsheet.

Data Quality Assessment

For each article the following was considered and documented:

a) Whether the study design or approach is appropriate to the research question, incorporating:

- whether the choice of outcome measure is valid and appropriate to the research question;
- whether there are any statistical issues in the analysis which may invalidate the study;
- whether the quality of reporting is adequate for incorporation within the technical paper;
- whether the study results are generalizable, within the remit of the technical paper.

b) The risk of bias in the study design/results.

Data Synthesis

The findings of individual eligible and quality-assured studies were then collated, compared, contrasted, combined and summarized. These results, together with the associated interpretations and conclusions generated from narrative and quantitative synthesis, and in accordance with the remit for the review, formed the technical paper.

APPENDIX 2

MECHANISMS OF ANTIMICROBIAL RESISTANCE TO DIFFERENT ANTIMICROBIAL GROUPS

A very brief summary of resistance mechanisms is provided below (van Hoek *et al.*, 2011):

The β-Lactams

Enzymatic inactivation: there are around 1000 different β-lactamases known to date, some are only able to inactivate a few substances while others can also inactivate third-generation cephalosporins (extended-spectrum β-lactamases or ESBLs) and β-lactamase inhibitors (e.g. clavulanic acid). The β-lactamases are widely dispersed across bacterial groups, they can be chromosomal or plasmid-encoded.

Acquisition of alternative pathways: as β-lactams inhibit several enzymes responsible for the synthesis of bacterial cell walls (known as penicillin-binding proteins or PBPs), the acquisition of substitute enzymes can render a cell resistant to many, or all β-lactams. Altered PBPs are behind penicillin-resistance in *Streptococcus pneumoniae*, acquired through transformation, either through accumulation of repeated point mutations but also through recombination between PBP genes from related streptococci species (Chambers, 1999).

Aminoglycosides

Enzymatic inactivation: approximately 100 acetyl-, phosphoryl-and nucleotidyl-transferases, which modify aminoglycoside molecules, rendering them inactive, have been reported. Most of these enzymes have a narrow spectrum (e.g. ANT(2")-I is only capable of inactivating gentamicin, tobramycin and kanamycin). Nevertheless, the bifunctional phosphoryl- and acetyl-transferase – found in Gram-positive cocci – can inactivate most aminoglycosides. Genes for these enzymes often reside in plasmids and transposons, and can be mobilized as gene cassettes between integrons.

Modification of target: recently, 16S rRNA methylases that modify the ribosome hindering the binding of aminoglycosides, have been reported in enteric bacteria, *Pseudomonas* spp. and Gram-positive cocci. Also ribosomal mutations can render the ribosome insensitive to aminoglycosides.

Tetracyclines

Active efflux: around 30 tetracycline genes, e.g. *tet*(A, B, C, D, E, G, H, J, K), encode a tetracycline-specific efflux pump, they are found both in Gram-positive and Gram-negative bacteria and are commonly encoded in transposons and integrons.

Protection of target: approximately ten genes, e.g. *tet*(M, O, Q, S, T), encode ribosome protection proteins that bind the ribosome, preventing the binding of tetracycline. In addition to enteric bacteria, these *tet* genes – particularly *tet*(M) are commonly found along macrolide resistance genes (see below '*Macrolides*' section) within the same transposon in Gram-positive cocci, and they can also be found in anaerobes.

Macrolides

Modification of target: several *erm* genes encode a 23S rRNA methylase that modifies the ribosome, hindering the binding of macrolides. This modification protects the ribosome from other chemically unrelated antimicrobials, such as lincosamides and streptogramins, hence called MLS_B phenotype, a clear example of cross-resistance. The *erm* genes are often found in mobile genetic elements, *erm*(B) and *tet*(M) are both within Tn*1545*, a conjugative transposon of streptococci.

Active efflux: several *mef* genes encode a macrolide-specific pump that reduces intracellular concentration of macrolides. Unlike *erm* genes, *mef* genes can only protect against macrolides, rendering an M phenotype.

Enzymatic inactivation: *mph* genes mediate inactivation of macrolides, these genes are found mostly in Gram-negative bacteria, limiting their clinical relevance. However, *vat* genes that also code for inactivating enzymes, are found in *Enterococcus* spp. and *Staphylococcus* spp.

Quinolones

Modification of target: Mutations in *gyr* and/or *par* genes encoding gyrase and topoisomerase IV enzymes, respectively, allow for these enzymes to complete their three-step DNA supercoiling process, even in the presence of quinolones. A single mutation can render a bacterial cell resistant to nalidixic acid, but two or more mutations are necessary to achieve resistance to fluoroquinolones (e.g. ciprofloxacin, enrofloxacin). Although these mutations are recessive in nature, supposedly limiting their ability to be horizontally mobilized, transfer through transformation has been documented in streptococci, as the newly acquired gene substitutes the old, wild-type one via recombination.

Active efflux: although a 'reserpine-sensitive' efflux mechanism of quinolone resistance in pneumococci was reported, this phenotype is rather a multidrug resistance one, mediated by unspecific efflux (see below).

Protection of target: a recently reported group of *qnr* genes encode a protein that protects topoisomerase enzymes from the action of quinolones. These genes, first reported as a unique plasmid-mediated quinolone resistance mechanism found in enteric bacteria, were then found in the chromosomes of many other organisms, along with related *mdp* genes of similar nature. These encode a low-level resistance to quinolones, often below the breakpoints for full resistance in the clinical setting.

Enzymatic inactivation: recently, a modified aminoglycoside-resistance enzyme, AAC(6')-Ib-cr, has been found to be capable of inactivating ciprofloxacin. This enzyme is rather common in clinical isolates of enteric bacteria with reduced susceptibility to ciprofloxacin.

Sulphonamides, trimethoprim

Acquisition of alternative pathways: sulphonamides inhibit dehydropteroate synthetase (DHPS) enzymes, while trimethoprim inhibits dihydrofolate reductase (DHFR) enzymes. By horizontally acquiring the genes for DHPS and/or DHFR variant enzymes that are not inhibited by these drugs, bacteria become resistant. Of particular importance is gene *sul*I, encoding one of such DHPS enzymes: this gene is part of the conserved region of class I integrons. Therefore, sulphonamides coselect for the entire genetic element, along with whatever other resistance genes have been integrated into the integron and viceversa.

Overproduction of target enzymes: mutants overexpressing DHPS and/or DHFR can overcome the inhibitory capacity of antifolate drugs at concentrations reached clinically, and become resistant.

Amphenicols

Enzymatic inactivation: chloramphenicol acetyltransferase enzymes, encoded by a variety of *cat* genes, inactivate chloramphenicol rendering the producing bacteria resistant to the drug. The *cat* genes have been found in Gram-positive and Gram-negative bacteria alike.

Modification of target: *cfr* genes encode for ribosomal methylase that modifies the ribosome so that florfenicol cannot bind, resulting in resistance. The clinical use of chloramphenicol is now very limited and florfenicol is now only used in veterinary settings. Nevertheless, *cfr* genes are relevant to public health as the methylase produced also protects the bacterial ribosomes from the action of linezolid, an

oxazolidinone antimicrobial considered as a 'last resort' drug against MRSA and VRE infections in humans. The *cfr* genes have been observed in linezolid-resistant clinical isolates worldwide.

Active efflux: *cml* and *flo* genes encode for specific efflux pumps, found mostly in Gram-negative bacteria.

Glycopeptides

Modification of target: glycopeptides bind to terminal D-alanyl-D-alanine residues of cell wall pentapeptide precursors, blocking the following steps of cell wall synthesis (transglycosylation and transpeptidation). The *van* genes alter the peptidoglycan synthesis pathways so that, instead of Dala-D-ala, there is D-alanyl-D-lactate or D-alanyl-D-serine. Clusters of *van* gene (five or more genes) are necessary to achieve glycopeptide resistance, hence the whole cluster must be horizontally transferred, likely through conjugation. Some *van* genes, probably originating from vancomycin-producing organisms, were transferred to *Streptomyces* species and then into Gram-positive cocci.

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