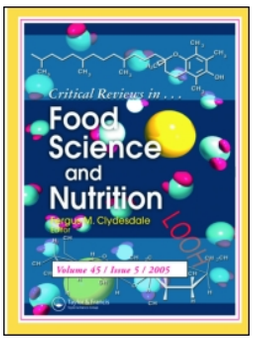

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Antimicrobial resistance: A global emerging threat to public health systems

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ABSTRACT

Antimicrobial resistance (AMR) became in the last two decades a global threat to public health systems in the world. Since the antibiotic era, with the discovery of the first antibiotics that provided consistent health benefits to human medicine, the misuse and abuse of antimicrobials in veterinary and human medicine have accelerated the growing worldwide phenomenon of AMR. This article presents an extensive overview of the epidemiology of AMR, with a focus on the link between food producing-animals and humans and on the legal framework and policies currently implemented at the EU level and globally. The ways of responding to the AMR challenges foresee an array of measures that include: designing more effective preventive measures at farm level to reduce the use of antimicrobials; development of novel antimicrobials; strengthening of AMR surveillance system in animal and human populations; better knowledge of the ecology of resistant bacteria and resistant genes; increased awareness of stakeholders on the prudent use of antibiotics in animal productions and clinical arena; and the public health and environmental consequences of AMR. Based on the global nature of AMR and considering that bacterial resistance does not recognize barriers and can spread to people and the environment, the article ends with specific recommendations structured around a holistic approach and targeted to different stakeholders.

KEYWORDS

Antimicrobial resistance; public health; food safety; surveillance networks; EU reduction strategy; international cooperation

Introduction

It is easy to forget what the world was like before the first antibiotic penicillin was discovered with observations by Alexander Fleming in 1928, when diseases like pneumonia and minor scrapes and infections often caused death due to septicemia. Another milestone in the history of development of antibiotics was the synthesis in 1935 by Gerhard Domagk of the first sulfonamide, prontosil, which was effective against streptococcus infections (Tortora, 2001).

Since then, the discovery and use of other class of antibiotics, or in general antimicrobials, have significantly contributed to the control of infectious diseases by reducing the associated mortality and morbidity rate in both humans and animals and to the advancement of medical technology (Arakawa, 2000). Also antibiotics provided major gains in life expectancy in the latter part of the last century. But what are antibacterial compounds? They are relatively small molecules with a low molecular weight (150–5000 Dalton) commonly classified based on their chemical origin into naturally occurring (antibiotics produced by bacteria and fungi, like penicillin), semisynthetic (e.g., methicillin), and synthetic substances (chemotherapeutic agents obtained by modifications of various natural compounds, like sulfonamides). A further classification is based on their biological effect on microorganisms and comprises two broad groups: (1) bactericidal agents that are able to kill bacteria, and (2) bacteriostatic agents that slow down or stop bacterial growth.

The term "antibiotic" has a more limited meaning compared to antimicrobial. In fact, the latter includes substances that act against all micro-organisms (bacteria, viruses, parasites, and fungi).

The most important development concerning the different classes of antibiotics took place in the period between 1949 and 1980. In the following period often defined as an "antibiotic discovery void," no new major antibiotics have been developed. More precisely, looking at the timeline of dates of discovery of distinct classes of antibacterials (as opposed to dates of introduction), there have been no successful discoveries of novel agents since 1987 (Silver, 2011). In fact only three new antibiotics for systemic administration active against Gram-positive bacteria have been developed after 1970. A new antibiotic (the first in nearly 30 years), teixobactin, has recently been discovered as having the potential to work against a broad range of fatal infections such as pneumonia, chronic infections caused by *Staphylococcus aureus* (MRSA, a so-called "superbug"), and tuberculosis (http://www.independent.co.uk/life-style/health-and-families/health-news/first-new-antibiotic-in-30-years-could-be-key-to-beating-superbug-resistance-9963585.html.).

Sadly, the public health benefits related to the use of penicillin and other drugs by placing under control the majority of infections throughout the past number of years were challenged by the emergence and spread of antimicrobial resistance (AMR). It is a fact that within one year of the widespread use of the penicillin, as warned by Fleming himself in 1945 in an interview with the New York Times, a significant number of *S.*

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aureus strains had become resistant to penicillin (Capita and Alonso-Calleja, 2013). The AMR became an unavoidable result of the fragile balance between bacteria and drugs with bacteria having infinitely more opportunities to gain resistance gene that human have to create new antimicrobials due to the infinitesimal generation time. (Huttner et al., 2013) In fact throughout almost four billion years of evolution, the microbial world has accumulated an enormous diversity of metabolic and protective mechanisms than can be mobilized in response to external aggression including antibiotics (e.g., antibiotic selective pressure) (Aminov, 2010). This evolutionary response to this "selective pressure" of antimicrobials is the base of the rapid development of resistance in bacteria and viruses. In response to the this selective pressure, bacteria by following a Darwinian process of natural selection, can survive, multiply, and produce a resistant progeny that progressively will replace the original not-resistant community.

Nowadays two main problems affect the efficacy of antibiotics. The first is that following the introduction of a new antibiotic, resistance to it will arise sooner or later. The second is the growing gap being recognized between the increased AMR and the development of new molecules. This means that the pace of discovery and development of new antibiotics is slower than the emergence and spreading of resistance mechanisms among bacteria, which can quickly respond to selective pressure and pass on the resistance genes when they replicate. One reason behind this asynchrony is that despite the clinical need for new antimicrobials, the pharmaceutical industry does not invest and create new drugs since the research and development required to bring one antimicrobial successfully to market might cost between $800 and $900 million and 10–15 years per approved agent (Monnet, 2005) and certainly the probability that the new molecule may become ineffective in the short-term might add a further motive of discouragement. Added to this is the increased regulatory conditions and strict price controls imposed by many governments (Norrby et al., 2005).

Thus, many in the pharmaceutical industry consider the research into new antibiotics less economically attractive and have prioritized long-term usage drugs for treating chronic illnesses such as hypertension, hypercholesterolemia, arthritis, diabetes, or cancer that are taken daily for the rest of a patient's life instead of antibiotics that are commonly taken for a short period of time (normally a few weeks), resulting in low sales.

The marked decline in industry research on novel antibacterial agents is reflected by the number (10 of the 15) of the largest pharmaceutical companies that since 1999 have significantly reduced or abandoned the antibiotic research programs (Projan and Shlaes, 2004).

AMR mechanisms

AMR is an expression of the ability of micro-organisms to counteract drugs commonly used to treat associated infections by developing mechanisms that render them resistant and allow for the transferring of resistant genetic traits to the community. Some of the fundamental bacterial mechanisms to resist to the effects of antimicrobials include: efflux pump (by removing antimicrobials penetrating the cell), enzymatic degradation of antibacterial drugs (e.g., by bacterial production of beta-lactamases), new metabolic pathways (e.g., synthesis of altered enzymes), alteration of bacterial proteins acting as antimicrobial targets (e.g., by modification of intracellular receptor of the antimicrobials, like ribosomal alterations), and changes in membrane permeability to antibiotics (e.g., outer membrane of Gram-negative bacteria like *Escherichia coli* confers a sort of impermeability to hydrophobic compounds such as macrolide antibiotics or to beta-lactams antibiotics). This resistance ability can be innate and intrinsically related to the general physiology or anatomy of a microorganism that confers resistance through the different mechanisms. In certain bacterial species (referred to as "insensitive" or "unsusceptible") intrinsic resistance is an inherent trait and is not affected by use (or misuse) of antibiotics (Capita and Alonso-Calleja, 2013). The most common way of acquiring resistance is through genetic changes such as mutation and uptake of genetic material by horizontal transfer from other bacterial strains. Despite mutations are relatively rare at about 1 per 10 (Monnet, 2005) to 10 (Capita and Alonso-Calleja, 2013) cells, there is a high probability of having huge increase in number of resistant bacteria due to the high replication rate. By horizontal transfer mechanisms, drug resistance genes can be spread from one bacterium to another through exchange of plasmids (extrachromosomal self-replicating DNA fragments transferred by conjugation, as occurs in Gram-negative bacteria) and virus (bacteriophages) transferred by transduction (as occurs in *S. aureus*). Plasmids can be transmitted both vertically and horizontally and are considered the most common and effective acquired resistance mechanism (Alanis, 2005). In particular, conjugative plasmids play an important role in the evolution of pathogenic bacteria because they are readily transmitted by horizontal transfer both between and within species (Johnson and Nolan, 2009).

Bacteria may also acquire resistance by transposons or integrons that can carry several resistance genes that differently from plasmids cannot replicate themselves, but can move within the genome. Integrons can be commonly carried in plasmids or be chromosomally integrated, as happens in *Salmonella enterica* serotype Typhimurium DT 104 (SCENIHR, 2009). Transposons can readily insert themselves into a broad host range of plasmids, thus providing functions to a large number of hosts; this facilitates the spread, in this case, of antibiotic resistance factors (Rowe-Magnus et al., 2002).

In recent times new mechanisms of resistance have resulted in the simultaneous development of resistance to several antibiotic classes creating very dangerous multidrug-resistant (MDR) bacterial strains, some also known as "superbugs." For instance, the MDR *Salmonella* Typhimurium phage type DT104, which is disseminated worldwide, like other major clones, carries the genomic Island 1 (SGI1) encoding resistance to different antibiotics. Because of its virulence and resistance gene repertoires, isolates with an SGI1 variant are a risk for rapid dissemination (Molbak et al., 1999; Mulvey et al., 2006).

Besides the well-known mechanisms of intercellular transfer of genetic material of the vertical type (mutations) or horizontal (transformation, transduction, conjugation), there are other ways of acquiring resistance that are related to the impact of the molecule on the physiology of the bacterial cell. In fact in recent years, an interesting concept emerged: the evolution of drug-resistance cannot be explained only with the selective

pressure exerted by the molecule "antibiotic" on the bacterial population, rather with a series of physiological changes of the bacterial cell. This phenomenon can be classified in two biological processes: molecular redundancy and molecular infidelity; the first is related to the ability of the molecule (environment) to induce changes (adaptation) in the physiological state of the cell that occur uniformly when the new environment is not lethal to the organism; the molecular infidelity make cells under stress more receptive to foreign DNA, and in addition can influence the frequency (high) of recombination and mutation (Heinemann, 1999).

Recent studies have found that antibiotic resistance and the different genetic mechanisms (e.g., co-selection and compensatory mutations) might affect the bacterial virulence and fitness (Willems et al., 2011; Beceiro et al., 2013). In recent years, clones derived both from animals (Kumar et al., 2011) and humans (Lozano et al., 2012) that are resistant to many antibiotics and carry virulence factors have spread globally, and they are considered highly successful or high-risk clones (Beceiro et al., 2013). This can be explained by the co-localization in the mobile genetic elements of the same genomic islands of virulence and antibiotic resistance genes. An example is the widespread dissemination in hospitals in five continents of *Enterococcus faecium* epidemic-virulent clonal complex 17 (CC17), which is characterized by ampicillin and quinolone resistance and by the presence of a putative pathogenicity island (Galloway-Pea et al., 2009). The OmpR-EnvZ operon system, which is encoded in *Salmonella* pathogenicity island 2, is also involved in the regulation of resistance and virulence (Johnson and Nolan, 2009).

AMR a growing public health threat

AMR is increasingly recognized by many international health organizations as a global public health issue and a threat to the modern health-care system that could hamper the control of many infectious diseases and dramatically set back the modern medicine. The growing worldwide phenomenon of AMR is generally associated to the "selective pressure" caused by the improper use, overuse, or misuse of antimicrobials in humans and animals (UN, 2001; Norberg et al., 2005). Infections by antibiotic-resistant strains are associated with a reduced quality of life, with metastatic bacterial infections, an increase in recurrence rates, chronicity, and future opportunistic infections with resistant organisms (Capita and Alonso-Calleja, 2013). This problem has been clearly documented by the rise of isolation of resistant human pathogens like *Salmonella* (Helms et al., 2004), *Campylobacter* (Helms et al., 2005), and vancomycin-resistant enterococci (Shay et al., 1995) (some of them leading to death) associated with a high frequency of therapeutic failures, increased risk of complications, worsening of pathological conditions, and death.

In the European Union, Iceland, and Norway, according to data from 2009, the European Centre for Disease Control (ECDC) estimates that AMR causes 25 000 deaths each year (ECDC, 2009a) with approximately 2.5 million extra hospital days. Similarly, in the same period in the USA and China, AMR caused 100 000 and 80 000 deaths, respectively (Wei, 2009; Spellberg et al., 2011).

Margaret Chan, WHO Director, when speaking about nosocomial infections due to resistant bacterial strains, stated that the "post-antibiotic era means, in effect, an end to modern medicine as we know it. Things as common as strep throat or a child's scratched knee could once again kill" (Chan, 2012).

The spread of AMR bacteria is considered an alarming public health threat, with a potential extent similar to global warming and other social and environmental threats. In the World Economic Forum's Global Risks 2014 report, 50 global risks (external) were analyzed in terms of their economic, environmental, geopolitical, social, and technological consequences, and classified according to their impact, probability, and interconnections. The impact and the probability of AMR were deemed as high as terrorism or climate change (http://www.weforum.org/reports/global-risks-2014-report). Recently became particularly worrying the potential of use multidrug-resistant agents included in bioweapons (Spellberg, 2008).

AMR was also discussed at the G8 Summit in June 2013, where science ministers identified it as the "major health security challenge of the 21st century" requiring intensive international collaboration.

In the USA the Center for Disease Control (CDC) recently released a report on AMR, entitled "Antibiotic Resistance Threats in the United States, 2013" where AMR is identified by its director Thomas Frieden as one of the nation's most serious health threats (CDC, 2013). The political will to reduce AMR is reflected by President Obama's five-year action plan to fight AMR where a $1.2 billion investment is being proposed for research and development of new antibiotics and the executive order signed in September 2014 instructing federal agencies to act on the National Strategy for Combating Antibiotic-Resistant Bacteria (http://www.whitehouse.gov/the-press-office/2014/09/18/fact-sheet-obama-administration-takes-actions-combat-antibiotic-resistan.).

Similarly in the UK the concern on the rise of AMR is reflected in the National Risk Assessment (NRA) report published on March 2015, that states that over the next 20 years, the numbers of infections complicated by AMR are expected to increase markedly. The report also foresees the possibility of an AMR outbreak that could cause around 200 000 people to be affected by a bacterial blood infection that could not be treated effectively with existing drugs and around 80 000 fatalities (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/419549/20150331_2015-NRR-WA_Final.pdf.). To tackle an epidemic outbreak caused by resistant pathogens the EU funded a "Platform for European Preparedness Against (Re-) emerging Epidemcis (PREPARE)."[1]

From data published in a report by Dame Sally Davies, Chief Medical Officer for England, it is estimated that in the UK 5000 people die due to a Gram-negative bacterium (such as *E. coli* and *K. pneumonia*) and in half of these cases, the cause is a bacterium resistant to antibiotics (Davies, 2013). The existence of the risk of death from an infection contracted during a minor surgical procedure proposes situations that are not dissimilar

[1]PREPARE is network for harmonized large-scale clinical research studies on infectious diseases, prepared to rapidly respond to any severe infectious disease outbreak, including those caused by resine pathogens (http://www.prepare-europe.eu/.)

to those related to health-care systems of the early 1900s. Under these conditions, the chances of therapeutic success in patients infected with multidrug-resistant strains are limited to a few of the latest generation antibiotics such as colistin and tigecycline.[2]

Today, there are fears for the effectiveness of so-called "CIA" (Critically Important Antibiotics) like fluoroquinolones, third- and fourth generation cephalosporins used for treating *Salmonella* spp. infections, and macrolides in cases of *Campylobacter* spp. infections (WHO, 2011). The emergence and rapid spread of carbapenemase producing gram-negatives such as extensively drug-resistant *Acinetobacter* spp. and enterobacteriacae producing New-Delhi metallo-protease-1 (Kumarasamy et al., 2010), *Klebsiella pneumoniae* carbapenemase, or oxacillinase 48 are a serious public health threat as these multidrug-resistant infections leave patients with few or no antimicrobial options (Silver, 2011).

Economic cost of AMR

AMR can lead to increasing costs and the destabilization of health systems. Patients suffering from AMR nosocomial infections (mainly bloodstream infections) or who become sick due to the consumption of food contaminated by resistant pathogens experience longer recovery and a higher frequency of septicemic infections and mortality (Angulo et al., 2004; Rice, 2009). In this situation, health-care costs are higher, due to extended hospital stays and the use of more expensive drugs. Moreover, there are higher risks of toxicity associated with new drugs, as well as a greater frequency of adverse drug reactions (ADRs) and collateral events (CDC, 2010).

Annual cost to the medical health sector in Europe resulted in health-care costs (direct and indirect) estimated to about €1.5 billion per year with €600 million in lost productivity (ReAct, 2012; White, 2011).

In the USA the annual societal cost-of-illness for AMR is considered to be roughly $55 billion (Smith and Coast, 2013) with a clinical impact higher than HIV infection (Roberts et al., 2009). Economists warn that the economic impact on the health-care system is expected to be higher since morbidity and mortality data related to drug-resistant bacteria are affected by limitations inherent to reporting systems (de Kraker et al., 2011).

In Europe, the situation is worsened by the economic and financial crisis that occurred in 2008 and afterwards, which might lead to less public health-care funding and investment for the development of new molecules by the pharmaceutical industry (Carlet et al., 2012). These socio-economic conditions inevitably impact on the epidemiology of AMR nosocomial infections. It should be also pointed out that economic cost to the health systems is estimated to be higher for developing countries because of the resources constraints of economical, health, and infrastructure systems affecting the availability and proper use of drugs (Sosa Anibal et al., 2010).

According to *The Independent Review on Antimicrobial Resistance* led by the economist Jim O'Neill the likely global cost of AMR by the year 2050, referred to increase morbidity and mortality will mean that the world population by 2050 can be estimated to be between 11 million (in the absence of AMR) and 444 million (if the problem is not tackled). The reduction in population and the morbidity would also impact on world economy by reducing the world Gross Domestic Product (GDP) by 2050 between 0.06 and 3.1%, depending on the scenario (http://www.rand.org/pubs/research_reports/RR911.html.).

In this situation, a winning strategy against AMR must focus on international cooperation; increased efforts at a national level; research of novel drugs; effective regulatory and public intervention; and reinforced communication between government, academic institutions, and the pharmaceutical industry, health-care professionals, veterinarians, and consumers.

AMR and nosocomial infections in the EU

To get an accurate picture of the extent and impact of AMR health-care associated human infections in the EU, it is worth to refer to the last ECDC report published in 2013. This report provides information on results of AMR surveillance conducted in EU member countries that, with some exceptions, show a deteriorating situation (ECDC, 2013). What emerges from the data for most microorganisms and antimicrobial agents is a geographic variation of prevalence of AMR isolates with a higher percentage of resistant and multiresistant isolates in south and south-eastern EU member states and the lowest percentages of resistance reported in the north. These differences might be related to geographical differences in infection control practices and in the use of antimicrobials.

E. coli *and* K. pneumoniae

Among the AMR pathogens monitored in the EU, the isolates of *E. coli* and *K. pneumoniae* steadily increased in the period from 2010 to 2013, with a trend significantly affecting more than a third of EU countries and EFTA countries (Iceland, Norway, Liechtenstein, and Switzerland). In the UK the spread of *E. coli* and *K. pneumonia* infections has tripled in the last three years, so as to represent the most frequent cause of infections acquired as a result of hospital admissions. Of particular concern for health authorities is the increasing percentage of *K. pneumoniae* isolates resistant to third-generation cephalosporins and to carbapenems, which are currently the last-line or last-resort antibiotics used for combating these bacteria. Increasing trends of carbapenem-resistant *K. pneumoniae* were significantly observed for Greece, Italy, and Romania. In Italy,

[2]Colistin and tigecycline are among the antibiotics that have become life-saving treatments for human patients suffering from different kinds of infections caused by multidrug-resistant bacteria. Colistin has been used in veterinary medicine for over 50 years. According to EMA in its response to a request of advice from the European Commission in 2013, there is no available evidence on the transfer of resistance to colistin from animals to humans. The Agency states that more research and surveillance on the subject should be done; recommends a responsible use in veterinary medicine (and should not be used for prophylactic use), and the strengthening of the surveillance for resistance to colistin. Tigecycline is not currently approved for use in animals even though there is evidence of use in dogs and cats. Any future marketing authorization in veterinary medicine should be based on a positive benefit-risk assessment, which would take into account the risk of transfer of resistance to humans. See http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2013/07/WC500146816.pdf.

the frequency of isolation for *K. pneumoniae* strains resistant to carbapenems has increased from 15% in 2010 to 34.3% in 2013.

Carbapenems are structurally related to β-lactams, used for the treatment of infections caused by multiresistant bacteria. The resistance to carbapenems, primarily attributable to the production of beta-lactamases, enzymes capable of hydrolyzing the amide bond of the ring with the production of inactive β-lactam derivative, is located on mobile genetic elements (plasmids) that facilitate inter- and intraspecies transfer (Carlet et al., 2012)). Of particular concern is that the drugs currently under development will not be effective against *K. pneumoniae*, for which recent studies have shown a 50% of mortality rate (Borer et al., 2009).

Despite carbapenem-resistant bacteria appear to be largely confined at present to the hospital setting, a jump to the community as seen with methicillin-resistant *S. aureus* (MRSA) is a real prospect for the near term.

The increasing trend throughout Europe also concerns *E. coli* resistant to third-generation cephalosporins, with the highest percentage in Bulgaria. A majority of the isolates were also extended-spectrum beta-lactamases (ESBL)-positive and showed resistance to additional antimicrobial groups. Of particular concern in Italy is a the increase of fluoroquinolone and cephalosporins resistant *E. coli* in 2013.

As documented in the ECDC report, the most alarming evidence of increased AMR is attributable to the multiple resistances to different antibiotics. In fact in some instances the use of one antimicrobial compound may give rise to the development of an MDR phenotype, as several resistance genes may be linked and transferred together on a mobile genetic element (Shea, 2004; Li et al., 2007).

This is the case in the EU for *E. coli* and *K. pneumoniae* isolates resistant to third-generation cephalosporins, fluoroquinolones, and aminoglycosides. In 2013, significantly increasing trends were observed for nine countries, with percentages of resistant isolates in the reporting countries ranging from 0% (Iceland) to 57.9% (Slovakia).

Methicillin-resistant S. aureus *(MRSA)*

In contrast to *E. coli* and *K. pneumonia*, the percentage of isolates of methicillin-resistant *S. aureus* (MRSA), which is one of the most important causes of antimicrobial-resistant healthcare associated infections worldwide, is currently stabilizing or decreasing in most European countries. In fact even though the decline of MRSA has been less pronounced in recent years compared with that observed during the first decade of the century, the trend for MRSA continues to decrease in nine out of 30 countries at European level. This decline can be explained by the success of the pathogen-specific vertical measures adopted to reduce human-to-human transmission that included screening before hospital admission and novel molecular diagnostic technique to rapidly detect the pathogen (Jarlier et al., 2010; Van Gastel et al., 2010; Afshari et al., 2012). However, MRSA is still considered a public health priority in Europe, as the percentage of MRSA among all isolates continues to be high (above 25%) in seven countries, especially in southern Europe. Italy is among the European countries with the highest levels of antibiotic resistance, particularly MRSA, with a frequency of 35.8% in the period relating to the year 2013, compared with a European average of less than 20%.

In the USA, MRSA strains are responsible for 80 000 human infections and 11 000 deaths annually (Mulvey et al., 2006; de Kraker et al., 2011). A study demonstrated that in case of bloodstream infection from MRSA, mortality is two or three times higher than in infection with nonresistant strain (Cosgrove et al., 2003). According to data released by WHO, bloodstream infections by MRSA and multidrug-resistant *E. coli* caused more than 8200 deaths in 31 European countries in 2007. These bacteria were responsible for more than 260 000 cases of bloodstream infections, which translate into more than 370 000 extra hospital days, for a total cost to health services of € 62 million

AMR, food-producing animals, and food safety

The major focus on human antimicrobial consumption and inadequate antibiotic stewardship in medical setting and the community as the main drivers for the development and spread of AMR can be explained by the availability of human data compared to animal data. However, it is generally acknowledged and heavily debated that the irresponsible and excessive use of antibiotics for the control of infections in animals, combined with the administration at sub-therapeutic doses (for prophylaxis or as growth promoters) in healthy animals through feed and water, has through the years contributed to the increased resistance of some pathogens that can propagate to humans (Hamer, 2002; Angulo et al., 2004; Phillips et al., 2004; Shea, 2004; Carnevale, 2005). One critical factor behind the emergence and selection of antibiotic resistance is that differently from human medicine where patients are treated individually with low dose of antibiotics, animal producers must use mass medication since it is impractical to treat individually each animal in a group that consists of hundreds to tens of thousands. Some researchers minimize the importance of the animal contribution to the emergence and spread of AMR in humans based on the fact that key foodborne human infections like *Salmonella* and *Campylobacter* are only a minor indication for antibiotic therapy (Capita and Alonso-Calleja, 2013). However, the unregulated and at times excessive use of growth promoters in animal husbandry and the use in animal treatment of common antimicrobials, particularly CIA's (e.g., quinolones, third and fourth generation of cepaholosporins), combined with the widespread horizontal transfer of antimicrobial resistant genes between commensal bacteria and human pathogens might corroborate the significance of animal sources as reservoir of resistance for human infections.

Use and overuse of antimicrobial in animal populations

What is beyond doubt is that large volumes of antibiotics used in food animals contribute to the problem of AMR, which appear to be much worse in the developing countries.

The World Health Organization (WHO), in a paper published as early as 2003 (WHO, 2003) and more recently in a report of 2009 (WHO, 2009), highlighted the public health consequences associated with the overuse and misuse of antimicrobials in animal productions. Moreover, in the 2009 *Global*

Report on Antimicrobial Surveillance it stated that there are "major gaps" in surveillance and data sharing related to the emergence of antibiotic-resistant foodborne pathogens and their impact on both animals and humans. One gap to fill is the amount of antibiotics consumed by food-producing animals worldwide each year.

Many countries report huge volumes of antibiotics used on animal husbandry. A recent study suggests that nearly half of the 210 000 tons of antibiotics produced in China are deployed in food animals (Wu, 2012). Data in the USA are particularly disheartening: more than 80% of all antimicrobials used were in food-production animals (Helena, 2011; Bowers, 2013; Van Boeckel et al., 2015b) and 70% of all antibiotics used are given for purposes other than treating infection (Mellon et al., 2001). According to recent estimate from the Animal Health Institute (AHI) growth promoters accounted for about 13% of the total amount of antimicrobials used (AHI, 2008).

One severe consequence associated to the excessive use is that 75–90% of antibiotics used in food animals are excreted, largely umetabolized, into the environment (Kumar et al., 2005) and the excreted drugs can persist in the environment creating an opportunity for resistance within exposed bacterial population (Rosenblatt-Farrell, 2009).

Mapping the antimicrobial consumption in livestock provides a baseline estimate of its global importance. Based on a new study published in the *Proceedings of the National Academy of Sciences (PNAS)* (Van Boeckel et al., 2015a) that provides the first global map (228 countries) of antibiotic consumption in livestock worldwide, the total figure will rise by 67% between 2010 and 2030, and possibly endanger the effectiveness of antimicrobials in humans. For both the 2010 and 2030 estimates, China and the USA are at the top of the list for their shares of worldwide animal antibiotic consumption and five countries, Brazil, Russia, India, China, and South Africa (the so-called BRICS), will experience a growth of 99% in antibiotic consumption.

Governments of China and India have certainly taken steps to curtail overuse of antibiotics by people, but they are lagging behind in regulating antibiotics in animal feed (Collignon and Voss, 2015).

In Europe, sales of veterinary antimicrobial agents have been monitored since 2010 through the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). From the corresponding report, which includes 26 EU countries and covers approximately 95% of the food-producing animal population in the EU/EEA area, there is a decline in the intensity of antibiotic use in animals, which fell overall by 15% between 2010 and 2012 (ESVAC, 2014). According to the recent joint report of EFSA, ECDC, and EMA, the consumption by these animals is much lower compared to humans in half of the countries (ECDC, 2015). In 2008, Kools et al. estimated that the most used antibiotic families in 25 EU member states in veterinary medicine were tetracyclines, bata-lactam antibiotics, and sulfamides (Kools et al., 2008).

Ban on growth promotants in animal feeds in the EU

Based on the concern that the wider use of antibiotics as feed additives in the long run can contribute to the development of resistant bacteria to drugs used to treat infections in animals and humans, the EU in an attempt to counteract this trend in 2006 imposed a ban on nontherapeutic feeding of antibiotics of human importance to farm animals. (http://europa.eu/rapid/press-release_IP-05–1687_en.htm.) A similar ban was imposed by the European Common Market in the mid-70s on tetracycline as growth promotant.

Before 2006, single member states prohibited on their territories the use of some antibiotics in animal feedstuffs. Examples are the bans of Avoparcin in Denmark in 1995 and Germany in 1996 arguing that this antibiotic produces resistance to glycopeptides used in human medicine; spiramycin was also prohibited in Finland in 1998 because of use in human medicine, and virginiamycin in Denmark in 1998 for the same reason. Currently, several countries including the USA permit the use of growth promotants in animal feeds with the consequences on the international trade (Castanon, 2007) with trading partners and competitors, such as the European Union, New Zealand, and South Korea, that have implemented restrictions on the importation of livestock and poultry products grown with antimicrobial drugs (Johnson, 2011). However, it has to be said that in the USA recent legislation seeked the phased elimination of nontherapeutic use in animals of "critical antimicrobial animal drugs" such as penicillin, tetracycline, macrolide, lincosamide, etc. that are used in human medicine (Bill Text, 2011).

Evidence of the link agricultural use of antibiotics-resistant infections in human

It is amply demonstrated that the antimicrobial resistant bacteria and their resistance genes are continually circulating in the soil, plants, food-producing animals, and throughout the food-chain.

The associations between antibiotic use in food animals and the prevalence of antibiotic-resistant bacteria isolated from those animals have been detected in observational studies as well as in randomized trials (Landers et al., 2012). In the Netherlands, the incidence of fluoroquinolone resistant *Campylobacter* spp. increased from 0 to 14% in broiler chickens, and from 0 to 11% in farm workers, following the introduction of enrofloxacin and saxifloxacin on poultry farms (Hamer, 2002). Some of the drug-resistant bacteria found in farm animals are responsible for zoonoses (e.g., *Salmonella, Campylobacter, E. coli*). Table 1 provides a list of relevant scientific papers related to the isolation of antibiotic-resistant pathogens in livestock.

Table 1. AMR pathogens in animal production.

Pathogen	Animal species, food	Reference
Staphylococcus aureus methicillin-resistant (MRSA)	Cow milk	Leonard and Markey (2008)
MRSA	Pigs	de Neeling (2007)
E. faecium	Poultry and pig	Sorenson et al. (2001)
Campylobacter spp. multiresistant	Cattle	Hakkinen (2010)
Campylobacter spp.	Bovine	Minihan et al., 2006
E. coli ß-lactam-resistant	Farm animals	Stefani et al. (2014)

These pathogens from food-producing animals can infect humans not only via food-borne routes through the consumption of contaminated or cross-contaminated foods of animal origin (Aarestrup et al., 2007; Overdevest et al., 2011) but also by routes such as water or environmental contamination, as well as through direct animal contact (Weese et al., 2006; Price et al., 2007; Van Loo et al., 2007; Soavi et al., 2010). The latter is particularly relevant for occupational workers or veterinarians that can be colonized and infected with resistant bacteria from animals. Despite this mode of transmission has not a relevant public health significance, the infected workers or their families might constitute a port-of-entry of resistant genes in the community and hospital environments as demonstrated by several studies (Lyons et al., 1980; Voss et al., 2005). There is also compelling evidence that commensal bacterial flora such as *E. coli* and *Enterococcus* spp. not only from the intestine of farm animals, but also of humans can form a potential reservoir of resistance genes that may be transferred between bacterial species, including organisms capable of causing disease in both humans and animals (Neidhardt, 1996; Van den Bogaard and Stobberingh, 2000; Winokur et al., 2001; Blake, 2003; Penders et al., 2013). These commensal bacteria are considered good indicators of the selective pressure exerted by the use of antimicrobials on intestinal populations of bacteria in food animals (EFSA, 2008a).

AMR in the environment

Recent studies have shown that the environment can host a broad spectrum of bacteria with the genetic determinants of resistance and that these, under appropriate conditions, can acquire pathogenicity. A Dutch study demonstrated that the proportion of bacteria containing drug resistance genes in the soil has increased significantly since 1940 (Knapp et al., 2010). Recently in the USA researchers tested microorganisms cultured from swabs of the New York subway stations and found that live, antibiotic-resistant bacteria were present in 27% of the samples they collected with one station producing a multidrug-resistant culture (Afshinnekoo et al., 2015).

From the environment (soil, water, and air), drug-resistant bacteria can directly or indirectly reach animals and humans by many routes including food crops of soils treated with antibiotic-containing manure (Dolliver et al., 2007; Cassone and Giordano, 2009). In regards to the link environment-resistant pathogens in humans, a case-control study conducted in Pennsylvania from 2005 to 2010 on more than 400 000 primary care patients revealed that the community exposure to crop fields where swine manure was used as fertilizer is a significant risk factor for both community- and health care-associated MRSA strains (Casey et al., 2013).

Antibiotic-resistant bacteria of animal origin have been observed in the environment surrounding livestock farming operations. In a study by the *United States Geological Survey*, antimicrobial residues were found in 48% of 139 streams surveyed nationwide, many of which were located downstream from animal agriculture operations (Kolpin et al., 2002). A study revealed that cattle located in close proximity to aquatic environments may disperse antibiotic resistant pathogens (*E. coli*) into such environments, which may lead to contamination of aquatic wildlife (Cissell, 2006). German authors examined 1000 samples of fruit, root, bulbous vegetables, and salads and have found that common bacteria on plants (*Enterococcus, Pseudomanas*) have in their genome resistance factors that can be transferred to intestinal pathogens (Schwaiger et al., 2011).

Graham et al. (2009) found that the bacterial flora present in flies collected from the areas around a poultry farm showed a resistance towards the same types of antibiotics used in the farm.

The fish sector, in particular the aquaculture farming, is particularly touched by the problem due to the extensive, unregulated, and indiscriminate use of antibiotics in much of the world (WHO, 2006). Despite there is much less data available on the public health impact of antimicrobial usage in aquaculture compared to other sources, it seems that the use of antimicrobials might contribute to the emergence and spread of AMR through the water, pond sediments, fish, and shellfish, and finally present a risk to public health (De Paola, 1995; Miranda and Zemelman, 2002; Joint FAO, 2006; Heuer et al., 2009). Also the integrated fish farming with the use of animal manure as fertilizers seems to favor antimicrobial-resistant bacteria in the pond environment (Petersen et al., 2002). Some authors suggested that historically the transfer and emergence of resistance have occurred faster from aquatic bacteria than terrestrial bacteria to humans (Sorum, 2006). Although the total quantities of antibiotics employed in aquaculture are estimated to be smaller than those used in land animal husbandry, there is much greater use of antibiotic families that are also used in human medicine (e.g., quinolones) (Marshall and Levy, 2011).

AMR in the food chain

There is undeniable evidence that farm animals (Harada et al., 2008) and different food of animal origin (White et al., 2001; Little et al., 2007; Oliver et al., 2009) contain abundant quantities of resistant pathogens.

However, it has to be noted that despite the massive evidence of environmental and animal source of AMR bacteria and the likely risk to humans, the answer to the question of whether antibiotic use in food-producing animals has led to the emergence, spread, and persistence of AMR in humans has been challenging and difficult to formulate conclusively due to the complexity of various routes of transmission between intraspecies (farm animal–human), species (human–human), and the frequent transfer of resistance genes among host bacteria (Wielinga and Schlundt, 2012).

Currently, there are several lines of evidence that link the therapeutic and/or nontherapeutic antibiotic use in livestock to increasing resistance in humans. These correlations were found in controlled human studies (as well as disease outbreaks) (Levy et al., 1976; Fey et al., 2000), timelines studies (Glynn et al., 1998; Engberg et al., 2001), microbiological studies (Shoemaker et al., 2001), and ecological studies (Campagnolo et al., 2002; Chapin et al., 2005). More recent and powerful epidemiological tools are the molecular genetic techniques that can demonstrate the same gene (or plasmid) in animal or human strains, even if the isolates are of different species (Kools et al., 2008), and provide useful information during outbreak investigations. Currently, phenotypic and molecular type data are the

best research tool to confirm that resistant isolates from animals or foods are indistinguishable from human health care isolates. Extremely useful are the attribution studies that are routinely undertaken in several countries like Denmark using surveillance data. The use of mathematical models allow to quantify the contribution of each major animal-food sources to human salmonellosis caused by antimicrobial resistant bacteria (Hald et al., 2007).

Transmission via foods is quantitatively the most important mode of transmission of antibiotic-resistant bacteria and resistance genes from the farm to the consumer (Van den Bogaard and Stobberingh, 2000). The consumer exposure to antimicrobial resistant bacteria via food of animal origin has been demonstrated by many studies. Alexander et al. found drug-resistant *E. coli* on surfaces of beef carcasses after evisceration and after 24 hours in the chiller and in ground beef stored for one to eight days (Alexander et al., 2010). Researchers in the USA isolated ciprofloxacin-resistant *Campylobacter* spp. from consumer chicken products (Gupta et al., 2004). In Italy, Normanno reported MRAS in cattle dairy products (Normanno et al., 2007). In another recent study of 200 samples of retail ground meat, 20% contained *Salmonella*. The majority of the *Salmonella* isolated (84%) were resistant to at least one antimicrobial, and more than half were resistant to greater than three antimicrobials. Disturbingly, 16% of the isolates were resistant to ceftriaxone, the drug of choice used to treat children with serious *Salmonella* illnesses (White et al., 2001).

Although correct food cooking kills bacteria, infection with resistant bacteria occur through improper handling before cooking. Several studies provided indirect evidence of the transmission of resistant genes from foods to humans by food handling and consumption. Researchers in the USA using genetic analysis showed that a high prevalence of ESBL-coding genes in retail chicken meat was the same as those found in rectal swab specimens from humans in the same geographic region (Collignon et al., 2013). Studies conducted in the USA by comparing the plasmid profiles of multidrug-resistant *Salmonella* Newport isolates from human and animal sources provided strong evidence that salmonella infections in 18 persons from four Midwestern states were linked directly to the consumption of hamburger meat from cattle fed subtherapeutic chlortetracycline (Holmberg et al., 1984). A control test, conducted in 2001 by Sorenson et al. with test volunteers, found the persistence of ingested glycopeptide-resistant *Enterococcus faecium* in the stools for up to 14 days after the initial ingestion of colonie chicken or pork meat (Sorenson et al., 2001). In 1985, scientists in Arizona found that multidrug-resistant *S. enterica* serovar Typhimurium isolated from most patients during an outbreak caused by consumption of raw, which included the death of a 72-year-old woman, were identical by plasmid analysis to the milk isolates (Tacket et al., 1985). In The Netherlands, 94% of a representative sample of chicken retail meat was contaminated with ESBL-producing *E. coli* isolates, of which 39% were also found in human clinical samples tested in 31 microbiological laboratories (Overdevest et al., 2011).

The American Center for Science in the Public Interest (CSPI) has documented 35 foodborne outbreaks between 1973 and 2009 in which bacteria resistant to more than one antibiotic had been implicated (Caroline Smith DeWaal et al., 2011).

A recent study comparing human and animal strains of *Clostridium difficile* indicates the potential transmission of these bacteria from farm animals to humans. *Clostridium difficile* was isolated from retail meat sold in Canada, Sweden, and the USA (Rodriguez-Palacios et al., 2007; Songer et al., 2009; Von Abercron et al., 2009) and ready-to-eat salads in Scotland (Bakri et al., 2009).

An interesting aspect that is currently under study and may shed new light on the broad resistance mechanisms of food pathogens is the ability of AMR bacteria to acquire similar resistance to environmental stresses associated with food processing (heat, refrigeration, pH, Aw, etc.). An increased thermal tolerance has been observed for S. *Typhimurium* DT104 strains, compared to susceptible strains of *S. typhimurium* and *S. enteritidis* (Walsh et al., 2005). Similarly, an acquired resistance to environmental stresses was detected in strains with mutations for resistance to fluoroquinolones. It is also demonstrated that several stresses (e.g., caused by a biocide or an antibiotic) are known to enhance DNA repair by activating a bacterial repair regulatory network system that controls the activation of integrons. Integrons are complex DNA fragments capable of integrating and regulating the expression of different antibiotic resistance genes, and thus able to confer multiple antibiotic resistances to bacteria (Erill et al., 2007; Guerin et al., 2009).

It is also well acknowledged that after sub-lethal treatments, injured bacteria may act to reduce the impact of stress by making phenotypic and genotypic adaptations (Wesche et al., 2009). If on one hand these stress-adapted cells may survive different steps of the food production process and hence present a challenge to the food industry, on the other hand, as demonstrated by several in vitro studies, the adaptation might be associated to the cross-protection against antibiotics (Aertsen et al., 2004; Rickard et al., 2004). More evidently further studies showed that processing stresses can increase the horizontal transfer of antibiotic resistance genes (Rodrigo et al., 2010). These events could favor the emergence and the spread of AMR via food consumption.

However, determining the role and the extent of the mild food technologies in the emergence and dissemination of resistant bacteria in foods and their impact on public health requires further research (EFSA, 2008b).

Of particular concern for the food industry is the widespread use of biocides such as disinfectants and preservatives that may lead to the emergence or proliferation of harmful bacteria that are resistant to both biocides and antibiotics. Based on many studies conducted in vitro the main involved mechanisms are referred to cross-resistance and the co-resistance. In particular the first implies the selection by a specific antimicrobial of genes that can expresses resistance to different antimicrobials (cross-resistance); in the second the expressed gene can be linked to another genes expressing resistance to other antimicrobials (co-resistance). These genes can be transferred among bacterial species via plasmids, transposons, and integrons. One study provides clear evidence of the association of use of biocides (e.g., quaternary ammonium compounds) to the expression and dissemination of beta-lactame resistance in Gram-positive bacteria (Sidhu et al., 2002). Other authors found an increased resistance to various antibiotics in *L. monocytogenes* and *S. enterica* strains after exposure to chemicals (e.g.,

acidified sodium chlorite, citric acid) (Alonso-Hernando et al., 2009). Potenski et al. (2003) described mutants of *Salmonella enteritidis* selected following exposure to chlorine or sodium nitrite, sodium benzoate, or acetic acid showing resistance to multiple antibiotics, suggesting the mar operon mutation was responsible for resistance.

A study by Gradel (2005) found a possible association between *Salmonella* persistence in poultry houses and resistance to commonly used disinfectants and a mutative role of the *mar* operon. Capita (2007) demonstrated that the use of acidified sodium chlorite may induce the selection in different serotypes of *Salmonella a* resistance against this biocide and a cross-resistance to various antibiotics.

However, despite the lack of precise data, in particular on quantities of biocides used, that make impossible to determine which biocides create the highest risk of generating antibiotic resistance, an assessment by *the European Commission Scientific Committee on Emerging and Newly Identified Health Risks* (SCENIHR) provides the first classification of biocides based on their potential to generate resistance. Surfaces agents like quaternary ammonium, phenolics, and metallic salts are considered highest-risk biocides (SCENIHR, 2009).

At an EU level the transmission of antibiotic resistance through the food chain is currently addressed by the EU's Seventh Framework Programme for Research and Technological Development (FP7) funded project EFFORT aimed at providing scientific evidence to inform decision-makers, the scientific community, and other stakeholders about the consequences of the transfer of AMR throughout the food chain (Ecology from Farm to Fork Of microbial drug Resistance and Transmission (EFFORT) is a research program aimed at studying the complex ecology of AMR and the complex interactions between bacterial communities, commensals, and pathogens in animals, the food chain, and the environment using a combination of epidemiological and ecological studies and newly developed molecular and bio-informatics technologies: http://www.effort-against-amr.eu).

The avoparcin case

Paradigmatic of the transmission of AMR from animal to humans is the relationship between a vancomycin-resistant *Enterococcus* (VRE) human infection and the use of avoparcin in livestock. Avoparcin is a growth promoter chemically related (and conferring cross-resistance) to the glycopetide vancomycin, which is a last resort drug for humans. In Europe the use of avoparcin in animals, mainly pig and chickens, caused frequent isolation of VRE in food-producing animals (Aarestrup, 1995; Hammerum et al., 2007), and humans (Kayser, 2003).

The differences between Europe and the USA in terms of the use of glycopeptides can shed some light on this. Avoparcin was banned as an auxinic in the EU until 1997. Denmark and Germany by applying the safeguard clause in the Directive on animal feed banned the antibiotic well before in May 1995 and January 1996, respectively. In the USA, where avoparcin has not been approved, on the basis of epidemiological evidence, the spread of VRE in humans was initially linked and confined to hospital environments instead of the general population due to its improper use in treatment protocols (McDonald et al., 1997). Contrastingly, in Europe, the rise of VRE was mainly associated with the use of avoparcin in animals. The Denmark case is emblematic of Avoparcin when it was first introduced in Denmark in 1988 as a growth promoter for pigs and poultry. The first evidence showing the avoparcin-AMR link was a 1995 survey in which researchers found VRE in 80% of chickens from conventionally producing farms (Avoparcin-using), whereas none were found in chickens from organic farms (Wielinga and Schlundt, 2012). Similar investigations conducted in other parts of the world confirmed this finding (Woodford, 1998). These investigations showed how the use of Avoparcin as a growth promoter can cause a high prevalence of VRA in chickens and likely serve as the origin of related human infections. In fact, in Denmark, the increase in VRA among humans was found as a result of therapeutic use in people to treat VRA infections or due to the consumption of contaminated meat (Aarestrup et al., 1996; Wegener et al., 1997). Once the use of avoparcin was discontinued, the prevalence of VRE among farm animals decreased. In fact in Denmark following the 1995 ban on avoparcin several investigations reported a decline in animal VRE (Bager et al., 1997; Pantosti et al., 1999). Also in humans a reduction in human carriage of VRE, following the ban of avoparcin, was demonstrated by two surveillance studies conducted in Germany and Belgium (Klare et al., 1999; Ferber, 2002).

The AMR monitoring program in the EU

According to Directive 2003/99/EC[3] on the monitoring of zoonoses and zoonotic agents, EU member states are obliged to monitor and report data on the resistance of strains of *Salmonella* and *Campylobacter* isolates from animals (pigs and broilers) and product thereof. Commission Decision 2007/407/EC has also established requirements for the harmonized monitoring and reporting of resistant strains of *Salmonella* isolated from poultry and pigs sampled in the framework of national control programs.

Data on drug-resistant strains isolated from animals, food, and human clinical samples[4] for the year 2013 are contained in a joint report annually published by EFSA and ECDC (Scientific report, 2015). The report provides data submitted by 28 EU MSs and is an important contribution to the work currently being carried out at the European level for addressing the problem of AMR. The majority of the results of susceptibility testing of clinical isolates of zoonotic bacteria from humans are interpreted using clinical breakpoints (clinical indicators)[5] to guide medical treatment of the patient. In animal and food isolates, the "microbiological" resistance was assessed using epidemiological cut-off

[3]The Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC covers: the monitoring of zoonoses and zoonotic agents; the monitoring of related antimicrobial resistance; the epidemiological investigation of food-borne outbreaks; and the exchange of information related to zoonoses and zoonotic agents.

[4]The EU legal framework for collecting data on human clinical samples is the Decision No 2119/98/EC setting up a network for the epidemiological surveillance and control of communicable diseases in the community.

[5]The generic case definitions for defining AMR refer to EUCAST (European Committee on Antimicrobial Susceptibility Testing) clinical breakpoints (www.eucast.org).

(ECOFF) values. The report suggests caution in making comparisons between isolates from different sources unless it is clear that methods and criteria for interpretation correspond.

Information on resistance is related to *Salmonella* and *Campylobacter* isolates from humans, food, and animals, whereas data on indicator *E. coli* and indicator enterococci isolates was related to only animals and food. The resistance patterns of strains isolated from humans, animals, and food, show differences between EU member states, and in general, compared to previous years, there has been no change in the resistance of monitored pathogens.

AMR Salmonella *in humans, food, and animals*

Strains of *Salmonella* spp. isolated from humans showed a high resistance to ampicillin, tetracycline, and sulfonamides, and low resistance to fluoroquinolones (ciprofloxacin and cefotaxine). Some of the investigated serovars exhibited very or extremely high multiresistance, such as monophasic S. *Typhimurium* 1,4,[5],12:i:- and *S. Kentucky*. In particular, it is the DT104 strain of *S. Typhimurium* distinguishable from other strains of *Salmonella* spp. by the presence of a genomic island (SgI1) that confers resistance to five antimicrobials (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines – ACSSuT).

Among *Salmonella* spp. isolates from animals (poultry, pigs, cattle), most MSs reported moderate or high to extremely high resistance to tetracyclines and sulfonamides and similar or slightly lower levels of ampicillin resistance. Resistance levels were generally higher in isolates from pigs and turkeys than from broilers, laying hens, breeding hens, and cattle. Overall, high to extremely high levels of resistance to fluoroquinolones (ciprofloxacin and nalidixic acid) were observed in *Salmonella* spp. isolates from fattening turkeys and broilers. Multiresistance was generally high in *Salmonella* isolates from broilers (56%), pigs (37.9%), and turkeys (73.0%), and low levels of "microbiological" co-resistance to ciprofloxacin and cefotaxime occurred in *Salmonella* from broilers, laying hens, and/or pigs (Belgium, Italy, Romania, and Spain). "Clinical" co-resistance to ciprofloxacin and cefotaxime was overall observed at the very low level of 0.3% in broilers and not detected in pigs and turkeys.

Salmonella spp. isolates from broiler and turkey meat showed the highest levels of resistance to ciprofloxacin and nalidixic acid. Ciprofloxacin resistance was reported in *Salmonella* spp. isolates from pig meat at low to moderate levels and was not detected among the relatively few isolates from meat from bovine animals. In most of the reporting MSs, "microbiological" resistance to the third-generation cephalosporins (cefotaxime) in *Salmonella* spp. from meat was either not discerned or detected at low levels.

High levels of resistance were found to tetracyclines, ampicillin, and sulfonamides among isolates, in particular in *S. Infantis* from broiler meat and *S. Typhimurium* (including the monophasic variants) from pig meat, resulting in extremely high levels of multiresistance (>70.0%).

AMR Campylobacter spp. *in humans, food, and animals*

In regard to *Campylobacter* isolates in human cases in the EU, very high occurrence of resistance to the clinically important antimicrobial ciprofloxacin was reported with more than half of *C. jejuni* and two-thirds of *C. coli* isolates being resistant. Similarly high levels of resistance were noted to ampicillin, nalidixic acid, and tetracycline, the latter for both *C. jejuni* and *C. coli*. Large differences in occurrence were observed between countries.

In animals, the highest levels of *Campylobacter* spp. resistance were seen for the fluoroquinolones (ciprofloxacin and nalidixic acid) and tetracyclines in all host species, while resistance to erythromycin and gentamicin was low or very low. Multiresistance was very low or not detectable in broilers (with the exemption of *C. coli*) but varied greatly in *C. coli* isolates from pigs ranging from 17.7 to 88.0%.

Campylobacter spp. isolated from broiler meat showed high resistance to ciprofloxacin, nalidixic acid, and tetracycline. The clear high levels of resistance in broilers, and the epidemiology of human campylobacteriosis infections with a large proportion deriving from handling, preparation, and consumption of broiler meat (EFSA BIOHAZ Panel, 2010a and 2011) (EFSA, 2010; EFSA, 2011), provide an example of how AMR in food and animals may impact on the availability of effective antimicrobial agents for the treatment of severe human *Campylobacter* infections. Although food is not currently considered to be a relevant source of MRSA (meticillin-resistant *S. aureus*) infection or colonization of humans (a low number of MSs reported the results of monitoring food for MRSA), MRSA was detected in meat from broilers, turkeys, pigs, and bovine animals. In live pigs the occurrence of MRSA showed a large degree of variation between MSs.

In summary, from the data collected by 28 EU member states, the resistance of bacteria to more than one antibiotic represents a common feature of *Campylobacter* and *Salmonella*, which are currently the most important pathogens responsible for foodborne infections in Europe. Campylobacteriosis is predominant in the EU, with more than 214 268 confirmed human cases in 2012, while *Salmonella* spp. is responsible for more than 91 034 cases reported in 2012. The phenomenon showed similar trends in previous years (The European Union, 2014).

National and international reaction to AMR

The AMR phenomenon has a cross-sectional nature involving different health professionals and requiring a holistic and integrated strategic approach based on the "one health" principle. If it is true that more than half of the antibiotics produced globally are used in livestock production, one of the likely effective measures for reducing the problem will be to focus on the farming sector[6]. This will not be an easy task, considering that even if we grant the association of cause (volume of drugs used in

[6]European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA), European Medicines Agency (EMEA), and the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR): Joint Opinion on antimicrobial resistance (AMR) focused on zoonotic infections. [http://www.efsa.europa.eu/it/efsajournal/doc/1372.pdf].

the farms) and effect (AMR), antimicrobials are still available without a prescription in some EU member states.

Despite the need for comparable monitoring data at the European level for relating the use of antimicrobials to AMR, research on this topic remains scant. A decisive step was recently taken by EFSA, EMA (European Medicines Agency), and ECDC, following a mandate from the EU Commission, yielding the first joint report on the integrated (food-producing animals–humans) analysis of the consumption of antimicrobials and the occurrence of AMR. This issue is further developed in section "AMR and OIE."

The availability of data pertaining to the classes and quantities of antibiotics used in each food producing animal species, time of treatment, aim of treatment (therapy, prevention, growth promotion), and the methods used for administration varies among the 28 EU member states. Only 19 out of 25 hold records pertaining to the quantities of antimicrobials used. Danish researchers are currently focusing on this type of data collection (prescription data) to understand how the different uses of antibiotics may affect the emergence of resistant bacteria. Similarly, the Netherlands collects data according to species at the farm level and produce digital data regarding drug traceability and the sensitivity tests required by farm veterinarians.

According to the action plan of the Commission on AMR[7] and the European Parliament's recent resolution,[8] the community objective is to reduce the prescription and use of antibiotics by implementing effective prevention measures, biosecurity, and vaccination. An opinion stated on 30 August 2012 by the Agriculture Committee of the European Parliament suggests that "by 2018 the use of antimicrobials for veterinary use in the European Union [should be] halved [when] compared to the data of 2012."[9]

In 2010, Denmark launched an initiative called "the yellow card" (DVFA, 2012) as a deterrent for reducing the use of antibiotics. Based on information gathered by VetStat, the *Danish Veterinary and Food Authority* (DVFA) identifies farmers and veterinarians with the highest use of antibiotics. This tool triggers a virtuous and competitive cycle aimed at a reduction in the use of antibiotics (http://www.whitehouse.gov/the-press-office/2014/09/18/fact-sheet-obama-administration-takes-actions-combat-antibiotic-resistan.).

One of the measures designed for reducing the use of antibiotics is so-called "decoupling," that is, separating prescribing from sales and stopping economic incentives offered to veterinarians for prescribing antibiotics. This proposal has been greatly debated and subjected to criticism from many pharmaceutical industries and professional associations. According to the Federation of Veterinarians of Europe (FVE), decoupling is not a solution and it is not supported by an impact assessment (http://www.fve.org/news/press.php?y=2012). In support of this, FVE cites the Netherlands and France that in the past number of years were able to cut-down the consumption of antibiotics over 50% and 30%, respectively, without needing to adopt decoupling.

Contrarily, in Denmark, for example, it is believed that the prescription and selling of antibiotics by veterinarians up to 1995 caused their massive consumption on farms and that the abandonment of this practice, along with other measures, has contributed to a significant reduction of more than 20–30% in the use of antimicrobials in animals.

EU strategies on AMR

In the early 90s, when AMR first appeared as a serious public health threat, the EU Commission launched a number of initiatives in human and veterinary medicine, food and feed, as well as in scientific research in an attempt to reduce human exposure to potential AMR bacterial strains. These actions included the phasing out of antibiotics for nonmedical use in animals[10] with a final step in 2006 banning the use of antibiotics as growth promoters in animal feed (See Ref. 30). Following on, similar measures adopted by the EU Commission covered a range of actions at the European and national level in the areas of data collection, surveillance, research, and awareness-raising and the funding of various projects pertaining to AMR through the Community Health Programme. The Commission under the EU's Seventh Framework Programme for Research and Technological Development, as well as the new framework program Horizon 2020 and the Innovative Medicines Initiative (IMI), financed more than 140 AMR-related research projects with a budget of euro 130 million (European Commission, 2015).

The Third Health programme 2014–2020 funding health initiatives, in order to facilitate access to better and safer health care for Union citizens, in one of the thematic priorities foresees specific measures to prevent AMR and control health-care associated infections (http://ec.europa.eu/health/programme/docs/factsheet_healthprogramme2014_2020_en.pdf.).

In 2011, the European Parliament, in a bid to address the public health threat of AMR, approved a resolution (See Ref. 41) in which it calls for the firm commitment of EU member state governments to implement AMR national strategies pertaining to the prudent use of antimicrobial agents, both in animals and human medicine. The Parliament expressed concerns that the previous European Commission action plans were not sufficient to contain the rising global threat of AMR resistance, and considered that new measures needed to be implemented as soon as possible. The Parliament called on the Commission to propose a legislative framework for action against AMR, by promoting initiatives and supporting dissemination of and information about:

- the prudent use of antimicrobial agents;
- the monitoring and surveillance of AMR;
- the need for research into, and the development of, new antimicrobial agents and alternatives;
- holistic approach; and
- international cooperation.

[7]Communication from the Commission to the European Parliament and the Council Action plan against the rising threats from Antimicrobial Resistance. COM (2011) 748. Disponibile su: http://ec.europa.eu/dgs/health_consumer/docs/communication_amr_2011_748_en.pdf.

[8]European Parliament resolution of 27 October 2011 on the public health threat of antimicrobial resistance: http://www.europarl.europa.eu/sides/getDoc.do?pubRef=-//EP//NONSGML+TA+P7-TA-2011-0473+0+DOC+PDF+V0//EN.

[9]Committee on Agriculture and Rural Development 2012/2041 (INI). http://www.epha.org/a/5378.

[10]Regulation (EC) No. 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition.

Table 2. Community action plan against the rising threats from antimicrobial resistance (2011).

Actions	
1	Strengthen the promotion of the appropriate use of antimicrobials in all EU member states.
2	Strengthen the regulatory framework on veterinary medicines and on medicated feed via the review package foreseen for 2013.
3	Introduce recommendations for prudent antimicrobial use in veterinary medicine, including follow-up reports, using the same approach as the 2002 Council Recommendation on prudent use of antimicrobial agents in human medicine.
4	Strengthen infection prevention and control within health-care settings.
5	Introduction of the new Animal Health Law, which will focus on the prevention of diseases, reducing the use of antibiotics and replacing current animal health provisions based on disease control.
6	To promote, through a staged approach, unprecedented collaborative research and development efforts to deliver new antibiotics to patients.
7	Promote efforts to analyze the need for new antibiotics in veterinary medicine.
8	Develop and/or strengthen multilateral and bilateral commitments for the prevention and control of AMR in all sectors.
9	Strengthen surveillance systems on AMR and antimicrobial consumption in human medicine.
10	Strengthen surveillance systems on AMR and antimicrobial consumption in animal medicine.
11	Reinforce and coordinate research efforts.
12	Survey and compare effectiveness research.
Areas	
1	Making sure antimicrobials are used appropriately in both humans and animals.
2	Preventing microbial infections and their spread.
3	Developing new and effective antimicrobials or alternatives for treatment.
4	Cooperating with international partners to contain the risks of AMR.
5	Improving monitoring and surveillance in human and animal medicine.
6	Promoting research and innovation.
7	Improving communication, education, and training.

To further strengthen its commitment, the Commission launched in November 2011 a five-year action plan against AMR, which covers seven areas and sets out 12 key actions both in the human and veterinary field (See Ref. 52) (see Table 2).

Essentially the action plan is based on a holistic approach involving all sectors and aspects related to the AMR such as: public health, animal health, food safety, consumer safety, research, nontherapeutic use of antimicrobials, etc.). In the veterinary field, it is recommended that the use of very important third- and fourth-generation antibiotics intended for human therapy be limited in terms of animal use.

Following the adoption on 11 December 2012 from the European Parliament of its own-initiative report on the Microbial Challenge-rising threats from AMR (http://www.europarl.europa.eu/oeil/popups/ficheprocedure.do?lang=en&reference=2012/2041(INI)#documentGateway), the Commission published an integrated road map and progress report on the implementation of the AMR five-year Action Plan[11]. With this document, which provides the operational objectives, concrete activities, and deadlines of each 12 key actions, the services of the Commission are further informing the European Parliament, Member States, and other stakeholders about the progress made on the implementation of the Action Plan.

Table 3 illustrates the framework for Action 2 of the Commission Action Plan road map that foresees the strengthening of veterinary medicine and medicated feed legislation to ensure that risks to human health arising from the use of antimicrobials in animals are adequately managed.

In the view of global nature of AMR, it is essential to develop international cooperation with organizations such as WHO, FAO, and OIE and bilateral collaborations with Third World countries. The Action Plan is furthering this global action with many initiatives contained in the Action 8 aimed at developing and/or strengthening multilateral and bilateral commitments for the prevention and control of AMR in all sectors.

To bolster the awareness of stakeholders regarding the prudent use of antibiotics and the public health consequences of AMR, European institutions organize a conference on antibiotic resistance in November of each year in Brussels, named "European Antibiotic Awareness Day" (EAAD). The EAAD, which is coordinated by ECDC, provides a platform for preexisting and planned national campaigns on prudent use of antibiotics and to support Member States by making available AMR communication materials that contain key messages targeted at the general public and at prescribers that can also be adapted and used at national level.

In addition, the Community Eurobarometer on AMR monitoring provides valuable information on antibiotics consumption in different countries.[12] In November 2013, a Special Eurobarometer Survey on AMR was published (http://ec.europa.eu/health/antimicrobial_resistance/eurobarometers/index_en.htm). As regards knowledge questions about antibiotics, the report showed that most Europeans (84%) are aware that unnecessary use of antibiotics makes them become ineffective. However, nearly half (49%) of Europeans do not know that antibiotics are ineffective against viruses, and over two-fifths (41%) do not know that they are ineffective against colds and flu. The main conclusions that emerged from the findings are related to: the need to target more effectively media campaigns at those who currently lack knowledge; and active involvement of doctors and pharmacies in EAAD as they play a key role in changing views and behavior.

The ECDC, EFSA, and EMA's antimicrobial surveillance activity

The EU Commission, in order to manage the public health risk of AMR and to evaluate the impact of interventions, relies on EFSA, ECDC, and EMA work, and supports wide-ranging and well-validated surveillance networks on AMR and

[11] Action Plan Against the rising threats from Antimicrobial Resistance: Road Map, available at: http://ec.europa.eu/health/antimicrobial_resistance/policy/index_en.htm

[12] European Commission: Antimicrobial resistance. Eurobarometer 338/Wave 72.5 - TNS Opinion & Social. [http://ec.europa.eu/health/antimicrobial_resistance/docs/ebs_338_en.pdf].

Table 3. Action 2: Strengthen the regulatory framework on veterinary medicines and on medicated feed.

Operational objectives	Concrete activities	Milestones/Deadline
To address Antimicrobial Resistance related to the use of veterinary medicinal products	Revision of the Veterinary Medicines Legislation	Veterinary Medicines Legislation: Commission proposal adopted in 2014 and sent to the EP and the Council Negotiations between the two institutions should take place at least until 2016
To address Antimicrobial Resistance related to the use of medicated feed	Revision of the Medicated Feed Legislation8	Medicated Feed Legislation: Commission proposal adopted in 2014 and sent to the EP and the Council

antimicrobial consumption for both humans (FWD-Net,[13] ESAC-Net[14], EARS-Net[15]) and animals (EFSA Scientific Network for Zoonosis Monitoring and ESVAC[16]) as part of the European Surveillance System (TESSy).

EFSA, through biological monitoring units[17], monitors and analyses AMR trends among animals used for food throughout 28 EU member states. These units are assisted by EFSAs Task Force on Zoonoses Data Collection, a pan-European network involving national representatives of each EU member state and of other countries, the WHO, and the World Organization for Animal Health (OIE).

EFSA also publishes scientific opinions on this matter (Scientific opinion, 2008; 2009a; 2009b) with the aim to harmonize the monitoring of AMR in food-producing animals, as well as to create comparable data obtained across EU member states and other EES countries for the provision of guidelines on minimal requirements for collecting and reporting (e.g., AMR in *Salmonella* spp., *Campylobacter* spp., *E. coli*, and *Enterococci*). These scientific reports, as well as other scientific opinions of EFSA, lead to the revision of the EU legislation on monitoring and reporting of AMR in food-producing animals and to the adoption by the European Commission of the Implementing Decision 2013/652/EU39, which entered into force on 1 January 2014.[18]

Based on the data collected by EU member states, EFSA in collaboration with the ECDC every year publishes the European Union Summary Report on AMR in zoonotic and indicator bacteria from humans, animals, and food, which includes data on AMR in *Salmonella* and *Campylobacter* infections in humans (See Ref. 43). EFSA also publishes reports on baseline surveys concerning the prevalence of AMR in specific animal populations in Europe, such as the presence of MRSA in pigs, and provides assistance to national authorities on reporting and monitoring activities.

In order to harmonize at European level the monitoring of human *Salmonella* and *Campylobacter* isolates and to ensure comparability of AMR in foodborne pathogens from human infections with that of food animals and foods, in March 2014 ECDC published a protocol where it recommends Member States to report quantitative AMR data (Minimal inhibitory concentration-MICs or zone diameters).

The new EFSA-ECDC-EMA joint report on strengthening surveillance systems on antimicrobial consumption

To strengthen surveillance systems on AMR and antimicrobial consumption in human and veterinary medicine, EFSA, ECDC, and EMA, following a mandate by the Commission and to further Actions 9 and 10 of the Commission Road Map on AMR, on 30 January 2015 published a joint report containing the results of the first integrated analysis of the possible association between the consumption of antimicrobial agents and the occurrence of AMR in both humans and food-producing animals at the European level (See Ref. 41). The three agencies based their work on 2011 and 2012 data, available from their five relevant EU monitoring networks based on information annually transmitted by the reporting countries.

From the main report findings for food-producing animals emerged that at EU level the consumption of antimicrobial agents[19] by these animals was lower or much lower than consumption by humans in half of the countries. For most of the evaluated combinations in food-producing animals, positive associations were observed between the consumption and occurrence of resistant bacteria, with the strongest of these instances noted in relation to *E. coli*., *Salmonella* spp., and *Campylobacter* spp. For humans, positive associations between consumption and resistance were observed for third- and fourth-generation cephalosporins and fluoroquinolones resistance in *E. coli*. For both food-producing animals and humans,

[13] Food and Waterborne Diseases and Zoonoses Network. Managed by ECDC.

[14] European Surveillance Antimicrobial Consumption Network (ESAC-Net) collects and analyses data on antimicrobial consumption from EU and EEA/EFTA countries, both in the community and in the hospital sector. The coordination of ESAC-Net was transferred from the University of Antwerp, Belgium, to the European Centre for Disease Prevention and Control in July 2011. Data are available from the EARS-Net interactive database from the ECDC website.

[15] The European Antimicrobial Resistance Surveillance Network (EARS-Net) as part of ECDC surveillance collects EU data on surveillance of AMR in human medicine. Data are available from the EARS-Net interactive database from the ECDC website as well as from detailed EARS-Net reports published each year in November.

[16] The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project is managed by EMA and collects information on how antimicrobial medicines are consumed in animals across the EU.

[17] The Evidence Management Unit (DATA Unit) plans, designs, and coordinates data collection including in the area of biological hazards. The Biological Hazards and Contaminants Unit (BIOCONTAM Unit) monitors and produces reports on foodborne zoonotic hazards, foodborne outbreaks, and antimicrobial resistance.

[18] Commission Implementing Decision 2013/652/EU of 12 November 2013 on the monitoring and reporting of AMR in zoonotic and commensal bacteria: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:303:0026:0039:EN:PDF. This Decision lays down the minimum requirements in Europe for the harmonized monitoring of the resistance to the most relevant antimicrobials from a public health perspective; combinations of bacterial species/food producing animal species/food; detailed rules for sampling, analysis of the isolates and interpretations of the results. The legislation includes also the requirements for the harmonized monitoring and reporting of ESBL-, AmpC, and carbapenemase-producing bacteria in certain animal populations and in certain food types.

[19] OIE defines "Antimicrobial agent" as: "a naturally occurring, semi-synthetic or synthetic substance that exhibits antimicrobial activity (kill or inhibit the growth of micro-organisms) at concentrations attainable in vivo. Anthelmintics and substances classed as disinfectants or antiseptics are excluded from this definition" (http://www.oie.int/index.php?id=169&L=0&htmfile=glossaire.htm).

positive associations were found between resistance in indicator *E. coli*. Positive associations were also noted for the consumption of macrolides in food-producing animals and the occurrence of resistance in human cases of *Campylobacter* spp. infection, and for consumption of tetracyclines and the occurrence of resistance in *Salmonella* spp. and *Campylobacter* spp.

The AMR in the global setting

AMR is being identified as a major health security challenge in the EU and this concern is shared internationally to contain the risks of spreading AMR from international trade and travel and via the environment. In view of the global nature of AMR the EU Commission's Action plan foresees international cooperation with international organizations such as WHO, FAO, and OIE and bilateral collaborations. Cooperation is a key condition, given that resistant bacteria are borderless and ineffective management by a single country could endanger others.

One of the instruments used to foster the international collaboration is the Global Health Security Initiative (GHSI).[20] The GHSI was launched in November 2001 as an informal, international partnership among Canada, the European Union, France, Germany, Italy, Japan, Mexico, the UK and the USA to strengthen health preparedness and response globally to threats of biological, chemical, radio-nuclear terrorism (CBRN), and pandemic influenza. The WHO serves as an expert advisor to the GHSI. GHIS recognizes that the prevention and control of risks to public health from AMR is exceedingly complex and requires global, active, and multisectoral collaboration within human health care, public health, animal health, food safety, food production, and environmental protection sectors.

AMR, WHO, and codex

To address the AMR threat, the WHO elaborated seven priority recommendations aimed at reducing the overuse or misuse of antibiotics in farm animals for the protection of human health (UN, 2011).

In December 2008, the WHO set up an Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) (http://www.agisar.org/) to support its efforts to minimize the public health impact of AMR associated with the use of antimicrobials in food-animals. The group comprises over 30 internationally renowned experts working in veterinary and food and public health disciplines relevant to AMR. The Antimicrobial Resistance Subcommittee (AMRSC) has the main objective of working towards global harmonization in order to make AMR surveillance data from different countries appropriately comparable. One of the main output of AMRSC is a guidance document published on 2012 that provides a step-by-step approach that countries need to follow to design programs for integrated surveillance of AMR, taking and using standardized and validated antimicrobial susceptibility testing methods and harmonized interpretative criteria (http://apps.who.int/iris/bitstream/10665/91778/1/9789241506311_eng.pdf).

The World Health Assembly adopted in May 2014 a Resolution that set up the WHO Global Task Force on Antimicrobial Resistance and renewed the collaboration with FAO and OIE to develop a global action plan under the WHO leadership. The Commission services are supporting and actively cooperating with the WHO in this mandate.

In 2011, the Codex Alimentarius adopted a guidance document on the risk assessment of AMR and on measures to be taken in human and veterinary medicine[21]. The guidelines provide science-based guidance on processes and methodology for risk analysis and its application to foodborne AMR related to nonhuman use of antimicrobial agents. Experts of the Commission, EFSA, and EMA actively collaborated in the drafting of this guidance.

AMR and OIE

In the veterinary sector the OIE (Office International des Epizooties), an intergovernmental organization responsible for animal health worldwide, published a document in 2003 containing international standards concerning AMR (OIE International, 2003). In a section titled, "Terrestrial Animal Health Code 2010" (Chapter 6.10, "Risk Assessment for AMR Arising from the Use of Antimicrobials in Animals"), it calls for the prudent use of antimicrobials in veterinary medicine and describes certain obligations that each operator in the supply chain of veterinary medicine is required to comply with. The OIE provides assistance to countries that are not yet able to apply the regulatory standards and that do not have the necessary infrastructures or human and financial resources.

In the most recent OIE general assembly held in March 2013, the need for international cooperation to ensure controls on the production, importation, marketing, distribution, and use of antibiotics was unanimously supported. The OIE standards on AMR were further developed with the participation of experts of the Commission, EMA, and EFSA in two ad hoc AMR groups to revise and update the chapters on AMR of the OIE Terrestrial Animal Health Code and to set up a global database on the use of antimicrobial agents in animals.

The Transatlantic Taskforce on Antibiotic Resistance (TATFAR)

Since 2009, the EU and USA have considered the mutual recognition of a program and activities related to AMR established during the Swedish presidency of the European Union in the form of a Transatlantic Taskforce on Antibiotic Resistance (TATFAR) (ECDC, 2009b) with the goal of improving cooperation in three key areas: (1) appropriate therapeutic use of antimicrobial drugs in medical and veterinary communities, (2) prevention of health care- and community-associated drug resistant infections, and (3) strategies for improving the

[20] The Global Health Security Initiative (GHSI) was launched in November 2001 as an informal, international partnership among Canada, the European Union, France, Germany, Italy, Japan, Mexico, the UK, and the USA to strengthen health preparedness and response globally to threats of biological, chemical, radio-nuclear terrorism (CBRN), and pandemic influenza. The World Health Organization serves as an expert advisor to the GHSI.

[21] Guidelines for Risk Analysis of Foodborne Antimicrobial Resistance (CAC/GL 77-2011).

pipeline of new antimicrobial drugs. Progress and the outcomes of the implementation of the recommendations related to the above key areas are summarized in a report published on 13 May 2014 (http://www.cdc.gov/drugresistance/tatfar/report.html). TATFAR is actually working on setting up an international working group to identify key knowledge gaps in understanding the association of the use of antimicrobial drugs in animals and the transmission to man and on the development of effective intervention measures to prevent this transmission, including the development of alternatives to antimicrobial drugs.

The EU is also contributing to the work against AMR by bilateral cooperation with China, Russia Federation, and developing countries in the fields of surveillance, promotion of prudent use of antimicrobials, awareness/communication, and health-care related infection prevention and control.

Recommendations

AMR is a global problem that needs to be tackled with solutions adapted to regional, national, and international levels by following a holistic approach and fostering global cooperation. Wide-ranging and highly coordinated efforts on several societal levels are imperative to slow the course of AMR.

One of the most important challenges ahead will be balancing competing interests and needs of different stakeholders. Therefore proposal of solutions and related recommendations must be targeted to the following subjects:

- Policy-makers and health authorities
- Human and veterinary health-care communities
- Consumers
- Industry

Policy-makers and health authorities

As shown above, the phenomenon of antibiotic resistance has a strong economical, healthy, and societal impact on humans and animal populations. On the human side, the AMR increases the rate of morbidity and treatment failure of human infections, prolongs hospital stays, and impacts negatively on health-care costs. In the veterinary field, the irresponsible use of antimicrobials in animals create an intermittent source of AMR pathogens that can spread in the environment and reach humans. Following the "One health" principle, policy-makers and health authorities should coordinate efforts that embraces human and veterinary fields in a holistic approach. They must acknowledge the need to:

- invest in preventive measures in human and animal practice to limit the need of using antimicrobials by requiring bio-security standards and vaccination plans;
- produce appropriate data to evaluate the economic and health impacts of AMR;
- ensure that risks are detected early enough, at global level (the Commission proposal for a Regulation on animal health creates a legal basis for the appropriate surveillance and early detection of listed pathogens in animals, among those, potentially, AMR ones) by rapid diagnostics that accelerate the identification and treatment of resistant pathogens;
- develop a system for collecting data deriving from official controls carried out by public veterinarians; and
- improve the comparability of data on resistance and use of antimicrobials both in human and veterinary medicine for strengthening risk management decisions and properly evaluating the measures taken. In particular the monitoring plans implemented by various countries should use standardized and validated antimicrobial susceptibility test and harmonized interpretation criteria to facilitate the comparison of the incidence of AMR cases among EU member states.

Human and veterinary health-care communities

The interventions in the veterinary and human sectors should be directed to:

- level the substantial differences in prescription policies between countries and corresponding behavior of prescribers either veterinarians or physicians;
- regulate the use of a specific molecule that might be affected by the market availability and price;
- collect accurate data on antimicrobial sales volume as an important step to monitor the AMR risk along the food chain and to support the related policies (The Commission proposal on veterinary medicinal products provides an obligation for collecting data on use of veterinary antimicrobials);
- implement effective drug traceability system and create a common database that contains consumption data aggregated according to therapeutic class, target species, dose, duration of treatment;
- implement effective enforcement actions;
- encourage the drug-surveillance activity by physicians, veterinarians, and pharmacists;
- promote a prevention culture both in human and veterinary medicine and discourage the self-prescription in veterinary and human medicine (particularly in case of flu and viral infection for which antibiotics are useless); and
- limit the veterinary use of CIAs (cephalosporins, third- and fourth generation, fluoroquinolones) for human health to situations that respond negatively to other antibiotics and only following the susceptibility test according to the underlying principle of responsible use " use antibiotic when necessary and as required."

Consumers

Data revealed by the special Eurobarometer on consumers' decision process behind consumption of antibiotics in the Member states indicate the need to engage civil society (associations, schools, etc.) by educational programs and media informative campaigns on AMR and the associated public health risk.

The objective of the AMR campaigns is to increase the awareness of consumers on the responsible use of antibiotics and on the current scale of AMR's threat for human health, animal health, and the environment. Furthermore, the general public must continue to be made aware that antimicrobials are a nonrenewable and endangered resource.

Industry

The pharmaceutical industry plays a crucial role in the ARM battle. It will not hold back in this important challenge because it represents one of the actors, both for research and for data on production and sales. These data are needed for designing an effective global system to fight AMR. It is certain that one of the most important challenges ahead will be balancing competing interests and needs, balancing the long-term need of keeping antimicrobials effective with the short-term need of treating infections in humans and animals. A novel public–private partnership should be explored to boost collaboration among experts from industry and academia and facilitate the antimicrobial discovery. Regulators will have to update the current system for marketing authorization and sales and financially support by using specific instruments in the research and development of novel antibiotics. In this context new economic models offering consistent incentives by reducing financial impediments to drug development should be explored as well.

Conclusions

Antibiotic resistance has been recognized as a global health problem and considered by major health organizations as the top health challenge in the 21st century. It is certain that the inappropriate therapeutic use and the nontherapeutic use of antimicrobials in animals is considered to be one of the drivers for the development of resistance in the human sector. AMR needs to be tackled on multiple fronts with the involvement of different stakeholders. The EU has proved to be in the upfront in tackling the public health and environmental consequences of AMR by developing an Action plan that provides a comprehensive and science-based strategy for the future. However, one of the main critical factors that affect the correct estimate of the magnitude and extension of the problem for both people and animals worldwide and the effective management is related to the scarcity of data on types of antibiotics used, amounts, animal species, and ways they are used (http://www.whitehouse.gov/the-press-office/2014/09/18/fact-sheet-obama-administration-takes-actions-combat-antibiotic-resistan.). Currently, Europe is one of the few countries with the best available data that will certainly be useful to prioritize regulatory interventions and practical action in the different sectors. Another problem is related to the need of more research to understand the dynamics and address the consequences of the incessant circulation of resistant bacteria and their resistant genes in the environment and along the different stages of the farm-to-fork transmission chain. It is recognized that besides the historical attention focused on a very small minority of resistant bacterial species that actually cause diseases in humans, there is a vast community of innocuous commensal and environmental bacteria that constitute a reservoir and an intermittent source of resistance genes that can propagate in animals and make their way directly or indirectly into human pathogens via food, water, and sludge and manure applied as fertilizers (Carnevale, 2005). Based on this evidence, we need better approaches that allow us to successfully restrict what and how antibiotics are used in these different environments. “One Health” appears to be a winning strategy to combat and reduce the ARM phenomenon but only if is consistently, vigorously, and effectively implemented by health professionals engaged in human and veterinary medicine.

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