

Antimicrobial resistance and residues in the EU: current situation and possible countermeasures, emphasis on *Campylobacter* and *Salmonella*

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REVIEW ARTICLE

Abstract

This review gives an overview on the prevalence of antimicrobial resistance in the food chain in the European Union. The main emphasis is on two important food pathogens, *Campylobacter* spp. and *Salmonella* spp. Furthermore, antibiotic residues reported in food commodities in the EU during 2008-2012, as well as the current legal framework regarding antibiotic use in the EU are discussed. In addition, the review also presents alternatives for the antibiotic treatment of food of animal origin.

Keywords: antibiotics, food pathogen, food safety

1. Introduction

Antimicrobials have been used to treat infectious diseases for over 50 years. Besides human clinical use their application involves farm animals, aquaculture, companion animals, and horticulture. Although antimicrobials are mainly used as medication, they are also applied as growth promoters at sub-therapeutic doses (Hao *et al.*, 2014; Marshall and Levy, 2011). Industrialisation of production and intensive farming has resulted in the (re)emergence of infectious diseases, and consequently to the increased use of veterinary antimicrobials. It has been estimated that the global use of antimicrobials in food animals will increase by 67%, from 63,000 tonnes to 106,000 tonnes between 2010 and 2030 (Van Boeckel *et al.*, 2015). Although antimicrobials have proven to be very effective against livestock pathogens, their use is associated with at least two adverse effects. Firstly, they are not specific towards pathogens but also kill commensal microbiota in livestock. Secondly, their extensive use has resulted in evolutionary adaptations in

microbes, resulting in wide-spread resistance (Capita and Alonso-Calleja, 2013; Laxminarayan *et al.*, 2013).

During the recent decades an increase of antimicrobial resistant (AMR) bacteria has been observed both in humans and animals (Anderson, 1968; Marshall and Levy, 2011; Schwarz and Chaslus-Dancla, 2001; Teuber, 2001; Wiedemann and Knothe, 1971). In response to antibiotic pressure, bacteria optimise their resistance mechanism towards multiple drugs to survive (resulting in multiresistance) (Mole, 2013). Microbial communities have also a potential to develop biocide-antibiotic cross resistances (Ortega-Morente *et al.*, 2013; Sheridan *et al.*, 2012). Consequently, contamination of the environment with pathogens resistant to antimicrobial agents is a serious threat, not only as a source of disease but also as a source of resistance genes that can easily spread to other bacteria in the environment (Marshall and Levy, 2011). Overuse and misuse of antibiotics in humans and animals is one of the major causes of AMR (Marshall and Levy, 2011).

Already in 1969 in the UK, the Swann report recognised that antibiotic growth promoters were contributing to the rise in multidrug-resistant *Salmonella*. However, there is still an ongoing debate about the role of antibiotic use in animals especially in the development of drug-resistant bacterial populations in humans (Cox and Ricci, 2008; Marshall and Levy, 2011).

The European Centre for Disease Prevention and Control (ECDC) estimates that each year AMR causes 25 000 human deaths and related costs of over € 1.5 billion in healthcare expenses and productivity losses (ECDC and EMEA, 2009). Until today, major foodborne bacterial pathogens, *Salmonella* spp. and *Campylobacter* spp., have persisted and remained as significant emerging foodborne pathogens (Koluman and Dikici, 2013; Newell *et al.*, 2010). According to the European Food Safety Authority (EFSA and CDC, 2014a), food-borne zoonoses cause more than 320,000 human infections in the European Union each year. Thus, the emergence of resistant bacteria, especially zoonotic ones, has a major impact on both human and animal health. This is a multidimensional problem that raises concerns among various stakeholder groups, including consumers and policy-makers, highlighting the need for an integrated approach to protect consumers from AMR related risks in the food chain by establishing appropriate preventive and control measures.

In recent years the EU has encountered several food crises. Therefore, the European Commission (EC) initiated EU strategic planning to deal with the development and spread of AMR. The EU has applied, across its member states (MS), a common policy and legislation covering antimicrobial use and monitoring and reporting programmes of zoonotic diseases and AMR status. EFSA, together with ECDC are responsible for collecting and analysing the relative scientific data deriving from MS. This review aims to give an overview on the prevalence of antimicrobial resistance in the food chain in the EU with main emphasis on two important food pathogens, *Campylobacter* spp. and *Salmonella* spp. Furthermore, antibiotic residues reported in food commodities in the EU during 2008-2012, as well as the current legal framework regarding antibiotic use in the EU are discussed. In addition, this review also presents alternatives for the antibiotic treatment of food animals.

2. Legal framework

The authorisation of any veterinary medicinal products in the EU is based on scientific assessments of the quality, safety and efficacy of the product as laid down in Regulation (EC) No. 726/2004 (EC, 2004). The scientific assessment is performed by the European Medicines Agency. Consumer safety issues related to consumption of potential residues of veterinary medicinal products via foodstuffs of animal

origin is included in the assessment, and, when necessary, maximum residue limits are established for the relevant residues in animal products. Imported animal products have to comply with the European legislation.

EFSA may be requested by the European Commission to carry out risk assessments related to residues of veterinary medicinal products, which are currently not authorised for use in the EU (EC, 2009). A notable exception in the legislation regarding antimicrobials is coccidiostats and histomonostats, which are substances intended to kill or inhibit protozoa *Eimeria* spp. and *Histomonas meleagridis*, respectively. Regulation EC1831/2003 states that 'certain substances with coccidiostatic and histomonostatic effects should be considered as feed additives for the purposes of this Regulation', although these are veterinary medicinal products (EC, 2003). This regulation also specifies that 'antibiotics, other than coccidiostats or histomonostats, shall not be authorised as feed additives'. Due to this regulation, coccidiostats and histomonostats actually form a category of feed additives, and can therefore be given to production animals much more freely than antibiotics. EFSA has assessed the use of these substances mainly in poultry and rabbits (EFSA, 2015)

During the recent years the EU has strengthened the surveillance systems on AMR. Harmonised data is needed for the better understanding of the epidemiology of AMR, for risk assessment and for the evaluation of the effectiveness of risk decisions and measures taken. The recently adopted Commission Implementing Decision of 12 November 2013 on the monitoring and reporting antimicrobial resistance in zoonotic and commensal bacteria (Decision 2013/652/EU; EC, 2013b) has been applied since 1st January of 2014. This legislation is based on e.g. the EFSA scientific report (EFSA, 2012) on 'Technical specification on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted in food chain' as well as other reports dealing with AMR. The current decision requires harmonised monitoring and reporting of the following bacteria: *Salmonella* spp., *Campylobacter jejuni* and *Campylobacter coli*, and indicator commensal *E. coli*, indicator commensals *Enterococcus faecalis* and *Enterococcus faecium*. Additionally, the legislation includes specific requirements for the harmonised monitoring and reporting of extended spectrum beta-lactamase (ESBL), ampC β -lactamases (ampC) and carpapenemase-producing bacteria in some foods of animal origin and other foods.

3. Critically important antimicrobials

The World Health Organization (WHO) has categorised antimicrobials used in human medicine based on criteria 1 and 2 in Table 1 (WHO, 2011a). Antimicrobials which meet criteria 1 and 2 are considered critically important antimicrobials (CIA; e.g. aminoglycosides, ansamycins,

carbapenems, and 3rd and 4th generation cephalosporins, macrolides and quinolones). Furthermore, WHO has prioritised the group of CIAs based on two criteria: applications 1.1, 1.2 and 2.1 (Table 1) (WHO, 2009). Drugs meeting both criteria and all three of applications 1.1, 1.2 and 2.1 are considered of the highest priority (Table 2).

Table 1. Categorisation and prioritisation of antimicrobials according to WHO (2009, 2011a,b).

Criterion 1	Antimicrobial agent is used as sole therapy or one of limited available therapy, to treat human disease Application 1.1: high absolute number of people affected by diseases for which the antimicrobial is the sole or one of few alternatives to treat serious human disease Application 1.2: high frequency of use of the antimicrobial for any indication in human medicine, since usage may favour selection of resistance	
Criterion 2	Antimicrobial agent is used to treat diseases caused by either organisms that may be transmitted via non-human sources or human diseases caused by organisms that may acquire resistance genes from non-human sources Application 2.1: greater degree of confidence that there are non-human sources that result in transmission of bacteria (<i>Campylobacter</i> spp.) or their resistance genes to humans (high for <i>Salmonella</i> spp., <i>Escherichia coli</i> and <i>Enterococcus</i> spp.)	

Table 2. Critically important antimicrobials of highest priority according to WHO (2011a).

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4. Veterinary drug residues in foods and food animals on the European market from 01/01/2008-31/12/2012

Over the surveyed 5 year period from 01/01/2008-31/12/2012, the European Rapid Alert System for Food and Feed (RASFF) recorded altogether 15,786 notifications in foods (<http://tinyurl.com/nvh6v3a>). Of these, 448 notifications concerned residues of veterinary medicinal products, making it the twelfth most common hazard category according to the RASFF database. Food imported into the EU accounted for the bulk of notifications while veterinary drug residues in foods from the EU member states triggered 72 RASFF notifications. Most RASFF notifications involved crustaceans and products thereof originating mainly from India and Bangladesh (Table 3). The main residues belonged to the nitrofurans group, chloramphenicol and leucomalachite green (Table 3). India, Bangladesh, China, Sri Lanka, Thailand and Vietnam accounted for 35% of all crustacean imports to the EU over the years from 2008 to 2010 and therefore continue to present a potentially serious problem (<http://tinyurl.com/p4qubfa>).

Problems in meat and products thereof formed the second most common category of RASFF notifications (Table 3). Brazil was responsible for 50% of all notifications over the five year period, largely because of ivermectin (antiparasitic drug) residues (Table 3). From 2008 to 2010, Brazil accounted for almost 14% of all imports of beef, while only one residue problems was encountered in beef from Argentina, which accounted for 40% of all imports (<http://tinyurl.com/p4qubfa>). Honey from China, Argentina, Mexico and Hungary formed the third most common category of RASFF notifications (Table 3), which together accounted for just over two-thirds of all imports to the EU between 2008 and 2010 (<http://tinyurl.com/p4qubfa>). Argentinian honey usually contained oxytetracycline residues, while Chinese honey was found to contain a wide range of antibiotic residues, frequently involving erythromycin or lincomycin (Table 3). Notifications concerning Mexican honey were restricted to streptomycin.

Problems in fish and fish products were responsible for the fourth most common category of RASFF notifications, with Vietnam accounting for the bulk of notifications (Table 3). In Chinese fish, nitrofurans and leucomalachite green residues were mainly found; the latter is an antimicrobial substance used to kill the fungus-like eukaryotic micro-organism, *Saprolegnia* (which infects fish eggs). Over the three years from 2008-2010, no RASFF notification was issued concerning veterinary drug residues in imports of swine, sheep or goat meat from any of the top ten most important exporting countries. For each commodity, the major ten exporters accounted for over 99% of all imports to the EU in that category (<http://tinyurl.com/p4qubfa>).

Table 3. Veterinary drug residues. Number of European Rapid Alert System for Food and Feed (RASFF) notifications, countries of origin and principal residues for the most affected commodities (>10 alerts) from 01/01/2008 to 31/12/2012 (<http://tinyurl.com/p4qubfa>).

Commodity	Main countries of origin (no.)	Principal residues (no.)
Total no. of RASFF notifications		
Crustaceans and products thereof		
190	India (87)	nitrofurans group (75) chloramphenicol (8) oxytetracycline (2) unknown (2)
	Bangladesh (69)	nitrofurans group (68) oxytetracycline (1)
	China (10)	nitrofurans group (8) unknown (2)
	Sri Lanka (6)	nitrofurans group (6)
	Thailand (5)	nitrofurans group (4) leucomalachite green (1)
	Vietnam (4)	chloramphenicol (2) nitrofurans group (1) cefalexin (1)
	Other countries (9)	chloramphenicol (5) nitrofurans group (4)
Meat and meat products (other than poultry)		
113	Brazil (57)	ivermectin (50) nitrofurans group (3) doramectin (2) albendazole (1) dihydrostreptomycin (1)
	China (10)	chloramphenicol (6) nitrofurans group (4)
	UK (10)	phenylbutazone (10)
	Italy (6)	chlortetracycline (2) sulfonamides (1) unspecified (3)
	Denmark (5)	chloramphenicol (5)
	Other countries (25)	chloramphenicol (6) prednisolone (4) sulfonamides (4) nitrofurans group (3) oxytetracycline (2) clenbuterol (1) dexamethazone (1) dihydrostreptomycin (1) metronidazole (1) ractopamine (1) unspecified (1)

Table 3. Continued.

Commodity Total no. of RASFF notifications	Main countries of origin (no.)	Principal residues (no.)
Honey and royal jelly 57	China (20)	erythromycin (7) lincomycin (4) chloramphenicol (2) streptomycin (2) siprofloxacin (2) metronidazole (1) nitrofurans group (1) sulfonamides (1)
	Argentina (6)	oxytetracycline (5) enrofloxacin (1)
	Mexico (5)	streptomycin (4) sulfonamides (1)
	Hungary (4)	nitrofurans group (3) sulfonamides (1)
	Other countries (22)	sulfonamides (10) tetracycline (4) oxytetracycline (3) metronidazole (2) nitrofurans group (2) chloramphenicol (1)
Fish and fish products 41	Vietnam (20)	nitrofurans group (6) leucomalachite green (4) chloramphenicol (4) malachite green (2) neomycin (2) ivermectin (1) victoria pure blue (1)
	China (4)	leucomalachite green (2) nitrofurans group (2)
	Germany (3)	malachite green (2) leucomalachite green (1)
	Other countries (14)	leucomalachite green (7) leucocystal violet (3) trimethoprim (2) chloramphenicol (1) enrofloxacin (1)
Animal feed (compound feeds, materials and pre-mixtures) 22	Belgium (5)	salinomycin (5)
	Ukraine (3)	chloramphenicol (3)
	Czech Republic (2)	bacitracin (1) salinomycin (1)
	China (2)	chloramphenicol (2)
	Other countries (9)	chlortetracycline (2) oxytetracycline (2) salinomycin (2) chloramphenicol (1) tetracycline (1) zilpaterol (1)

Veterinary drug residues in animal feed are a potential source of prophylactic levels on antibiotics and are subject to control under Regulation (EC) No. 1831/2003 (EC, 2003). Residues were found in a variety of feeds, feed supplements and feed materials, especially complementary and compound feeds for poultry, rabbits and fish. In addition to residues listed in Table 3, a number of unauthorised substances were found while the prohibited substance chloramphenicol was found in shrimp feed from Singapore, vitamin A pre-mix/supplement from China and milk-based products from the Ukraine. The prohibited substance zilpaterol was also found in broiler feed from Poland.

5. Overview on antimicrobial resistance data from Europe

In the recent years, joint reports of EFSA and ECDC have been published about the occurrence of zoonotic infections and agents in humans and animals. The two most commonly reported zoonotic infections are campylobacteriosis and salmonellosis (EFSA and ECDC, 2011, 2012, 2013, 2014a). In Europe, in 2012, Enteritidis and Typhimurium were the two most commonly reported *Salmonella enterica* serotypes, representing 41.3 and 22.1%, respectively, of all confirmed human cases (EFSA and ECDC, 2014a).

According to the recent EFSA and ECDC (2014b) survey, the antimicrobial resistance was commonly detected in isolates of *Salmonella* and *Campylobacter* from human cases as well as from foods of animal origin and other foods. This was also reported for indicator (commensal) *E. coli* isolated from animals and foods. The occurrence of resistance in *Salmonella* isolated from human cases was high for ampicillin, streptomycin, tetracyclines and sulfonamides and moderate for nalidixic acid, with high levels of multi-drug resistance observed in isolates obtained from certain countries. Furthermore, data show that antimicrobial resistance in *Salmonella* spp. (Figure 1) and *Campylobacter* spp. (Figure 2) isolated from humans, foods of animal origin and other foods is a frequent concern, although levels of resistance are subject to strong variations across member states (EFSA and ECDC, 2013, 2014b). Multi-resistance to antimicrobials has become a serious public health problem (Doyle *et al.*, 2013; WHO, 2011b).

EFSA and ECDC surveys (2013, 2014b) as well as recent reviews (De Jong *et al.*, 2011; Silley *et al.*, 2011) have recommended reporting both clinical antimicrobial resistance and decreased susceptibility. For instance, for fluoroquinolones, not only clinical breakpoints are relevant, but for monitoring purposes it is also necessary to address the population of isolates with decreased susceptibility, yet remaining clinically responsive to the antibiotic. Clinical breakpoints and epidemiological breakpoints are needed for monitoring purposes (Silley *et al.*, 2011). According to EFSA and ECDC (2013) there was a lack of standardisation of

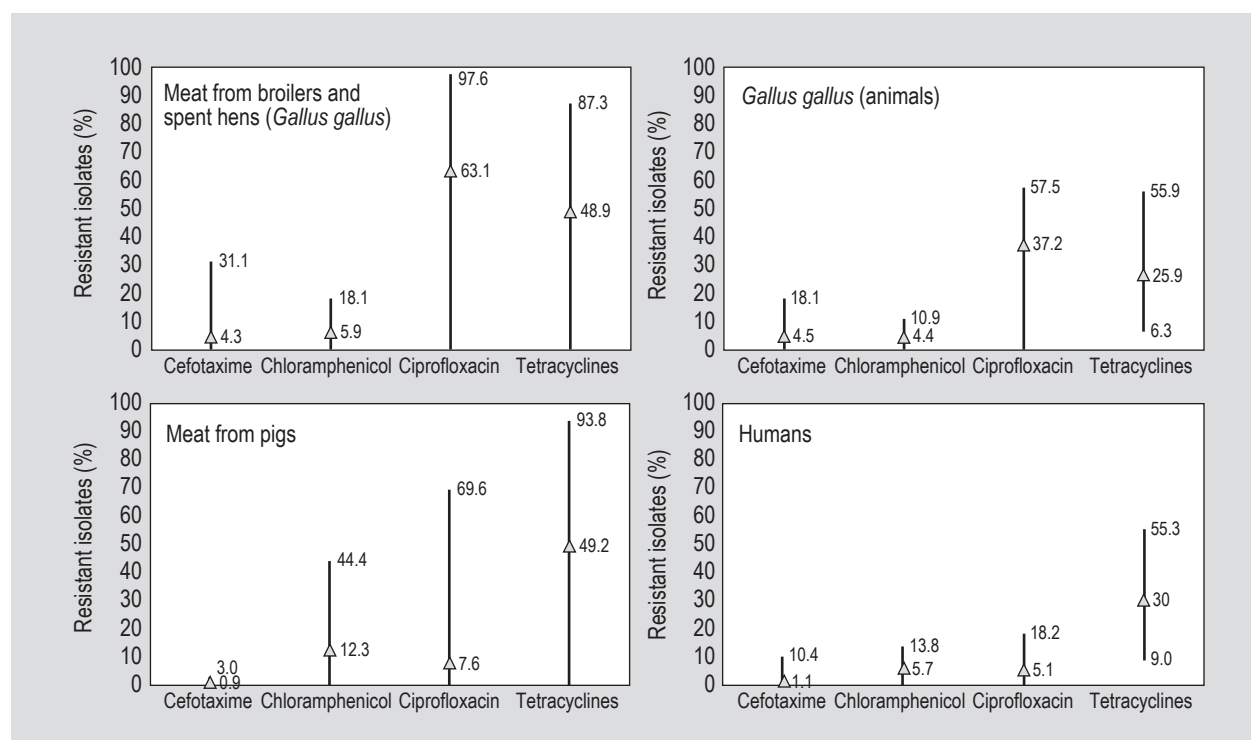


Figure 1. Antimicrobial resistance in *Salmonella* spp. isolates from different sources in EU in 2012 (data according to EFSA and ECDC, 2014b, using harmonised epidemiological cut-off values). Data for meat from broilers and spent hens (*Gallus gallus*) includes data reported by 12 member states (MS). Data for animals includes breeding and laying hens and broiler flocks of *Gallus gallus* reported by 16 MS. Resistance levels in *Salmonella* spp. isolates from human cases were reported by 18 MS. Data for humans by using clinical breakpoints from all non-typhoidal serovars. Columns present highest, lowest and average percentage of resistant isolates reported in the MS.

antimicrobial susceptibility testing methods and interpretive criteria both between and within countries. Most countries use clinical breakpoints for the interpretation of test results as provided by the Clinical and Laboratory Standards Institute (CLSI) or a combination of clinical breakpoints from CLSI and the European Committee on Antimicrobial Susceptibility Testing, depending on the antimicrobial. A few countries used other criteria such as epidemiological cut-off values (EFSA, 2013; Silley *et al.*, 2011). The recent EU legislation (2013/652/EU; EC, 2013b) and its technical annex provide more harmonised rules for all member states for the monitoring of AMR in animals and in foods.

6. Resistance of *Salmonella* spp. and *Campylobacter* spp. to CIAs

Salmonella and *Campylobacter* are the most common causes of bacterial foodborne diseases in industrialised countries and an increasing prevalence of antimicrobial drug resistance has been recognised in them (Capita and Alonso-Calleja, 2013; Doyle *et al.*, 2013; EFSA and CDC, 2014b). Studies have shown that infections with resistant *Salmonella* spp. and *Campylobacter* spp. can result in higher mortality compared to infections caused by susceptible strains (EFSA, 2013). Therefore, special

attention has to be given to the reduction of the prevalence of these pathogens in food products and to the presence of antimicrobial resistance genes in these strains. Based on the WHO prioritisation of CIAs particular emphasis is given to quinolone and cephalosporin resistance (third- and fourth-generation) in *Salmonella* spp., and quinolone and macrolide resistance in *Campylobacter* spp. (ECDC and EMEA, 2009; Hopkins *et al.*, 2010; WHO, 2011a,b).

Quinolones

According to a report by the WHO (1997), the use of fluoroquinolones (antibacterials that prevent bacterial DNA from unwinding and duplicating) in poultry has caused a dramatic increase in the incidence of resistant strains of *Campylobacter* spp. in poultry and subsequently in humans (McDermott *et al.*, 2002; Nachamkin *et al.*, 2000). The first resistant strains of *C. jejuni* in Europe were discovered during the 1980s (Nachamkin, 2002). The removal of fluoroquinolones from the battery of veterinary medicines has not entirely eliminated the presence of resistant *C. jejuni* and *C. coli* in animals and foods of animal origin (Smith and Fratamico, 2010), on the contrary it seems that such resistance is even increasing (Ge *et al.* 2013; Wimalaratna *et al.*, 2013).

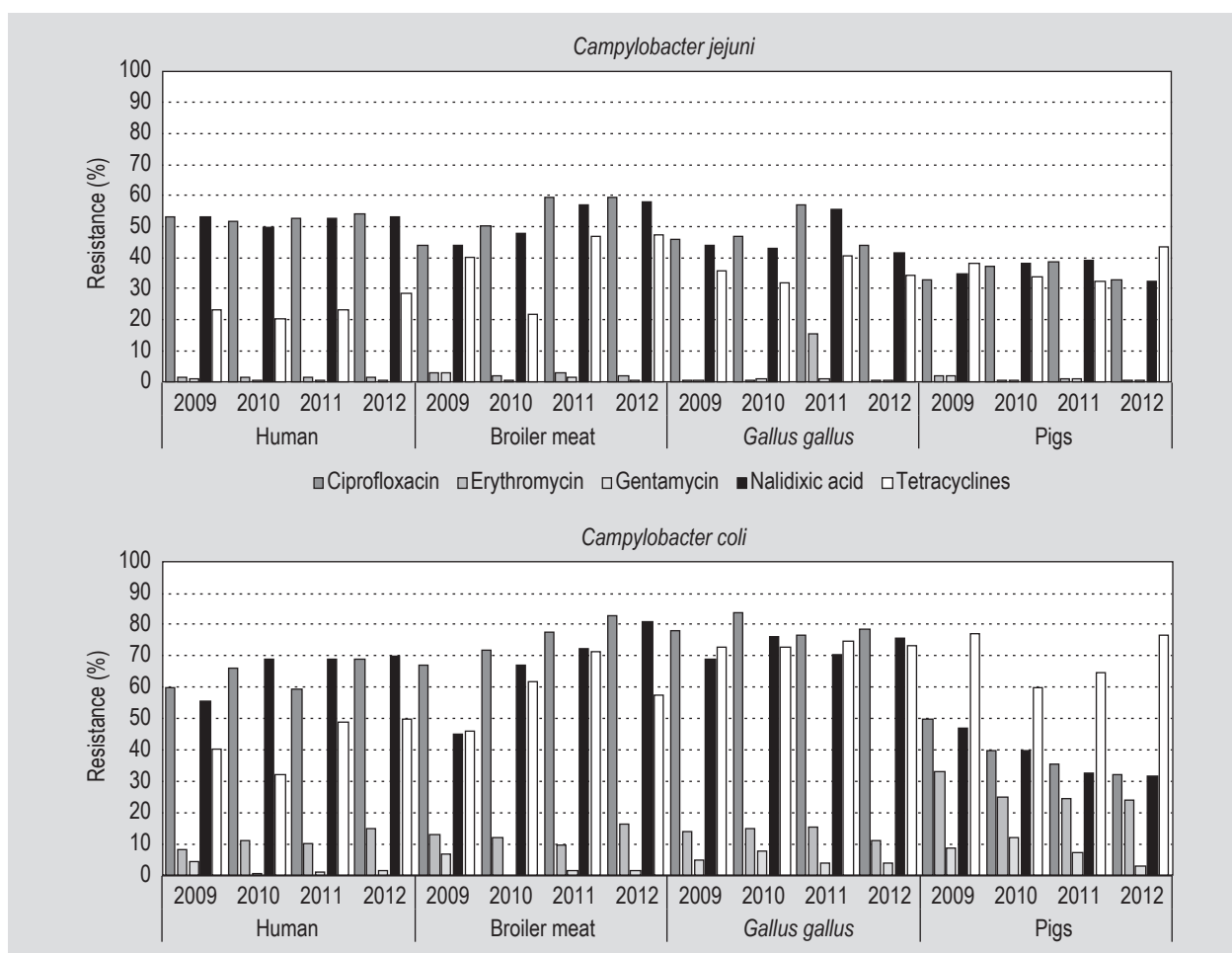


Figure 2. Antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* in EU for humans using clinical breakpoints and for other sources using harmonised epidemiological cut-off values. Data according to EFSA and ECDC (2011, 2012, 2013, 2014b).

Fluoroquinolones inhibit the growth of bacteria by binding to bacterial DNA gyrase and DNA topoisomerase IV. These enzymes are associated with bacterial transcription, replication, and chromosome condensation and segregation (Smith and Fratamico, 2010). Resistance to fluoroquinolones has developed primarily as a result of mutations in the *gyrA* gene (McDermott *et al.*, 2002; Zhang *et al.*, 2003). Among such mutations, Thr86Ile is the most prevalent (Pérez-Boto *et al.*, 2014). Together with the mutation of DNA gyrase, the presence of activated efflux pumps (ejection mechanism that allows toxic substances such as antibiotics to be transferred from the bacterial cytoplasm into the environment) has been detected in strains resistant to fluoroquinolones (Kovač *et al.*, 2014; Webber and Piddock, 2003).

High level of resistance to fluoroquinolones in *Salmonella* has mainly been explained by the combination of two major resistance mechanisms, multiple target gene mutations and active efflux mediated by AcrAB-TolC (Hur *et al.*, 2012). Yamasaki *et al.* (2013) also concluded that for AcrB multidrug efflux-pump and bulkiness of

lipopolysaccharide core oligosaccharides are essential for intrinsic antibiotic resistance in *S. enterica*. Studies conducted in various countries have proved the connection between the development of resistance in strains isolated from humans and animals and the beginning of use of fluoroquinolones in veterinary medicine. Broilers are now considered as the most important source of fluoroquinolone resistant *Campylobacter* (Eurosurveillance editorial team, 2014; Pérez-Boto *et al.*, 2014). According to EFSA and CDC (2014b), extremely resistance to ciprofloxacin was commonly observed in *C. coli* isolates from broiler meat and broilers (*Gallus gallus*), with 82.7 and 78.4%, respectively, with somewhat lower levels were seen in *C. jejuni*, with 59.5 and 44.1%, respectively.

Resistance to quinolones in *Salmonella* isolated from animals and foods of animal origin has increased in many countries around the world during the last years (Doyle *et al.*, 2013; Gyles, 2008; Hur *et al.*, 2012). However, in the EU, there are differences between countries regarding the serovars isolated, their host animals and subsequent food products (EFSA, 2014b; Maka *et al.*, 2014). In their

European Antimicrobial Susceptibility Surveillance programme study, de Jong *et al.*, (2011) reported, 2005–2006, only 6.1 and 4.0% of bovine and porcine isolates, respectively, were resistant to ciprofloxacin, which was much less than chicken isolates (47.8%). Likewise, Maka *et al.* (2014) reported that *Salmonella* spp. strains isolated from poultry products were resistant to a wider spectrum of antibiotics than strains of other origins. According to the recent EFSA and ECDC report (EFSA and CDC, 2014b), in food and animal isolates, the highest occurrence of resistance to ciprofloxacin was noted in *Salmonella* spp. isolates from fattening turkey, broiler meat and fowl, from 37.3 to 86.2%. Chen *et al.* (2004) characterised multiple-antibiotic resistant *Salmonella* serotypes from retail meats originating from the USA and China. They observed that 11% of the Chinese isolates were resistant to nalidix acid and had decreased susceptibility to ciprofloxacin. *Salmonella* Kentucky strain ST198 resistant to ciprofloxacin has emerged during the last years (Doyle *et al.*, 2013; EFSA, 2014b). It was first described by Le Hello *et al.* (2011) in humans and since that it has been isolated from broilers in Ireland and turkey meat in Poland (Wasył and Hoszowski, 2012). In Poland, 89% of the turkey isolates (n=72) were resistant to both nalidixic acid and ciprofloxacin (EFSA and ECDC, 2013).

Macrolides

Erythromycin, a bacteriostatic antibiotic from the macrolide group, is usually the drug of choice for treatment of campylobacteriosis in humans. Sensitivity of poultry *Campylobacter* strains to macrolides has been investigated on several occasions, with variable results (Hariharan *et al.*, 2009; Wirz *et al.*, 2010). The majority of the *Campylobacter* strains were either highly susceptible (Hariharan *et al.*, 2009) or fairly resistant to erythromycin (Smole-Mozina *et al.*, 2009). The effect of macrolides and azalides on bacterial cells is based on the interruption of protein synthesis, as they bind to the P-site 50S of ribosomal subunit and block the activity of peptidyl-transferase (Payot *et al.*, 2006). *Campylobacter* strains exhibit two different phenotypes with regards to erythromycin resistance: high-level resistance and low-level resistance (Caldwell *et al.*, 2008). Resistance to macrolides is usually associated with the mutation A2075G in the 23S ribosomal RNA gene (Kurincic *et al.*, 2007; Lehtopolku *et al.*, 2011). Other mutations found in the 50S ribosomal subunit encoding proteins L4 and L22 do not appear to be linked to the high-level erythromycin resistant phenotype (Corcoran *et al.*, 2006). As with fluoroquinolones, the presence of activated efflux pumps may reduce the sensitivity of some *Campylobacter* strains to macrolides, as well as decreased membrane permeability due to mitochondrial outer membrane permeabilisation (Corcoran *et al.*, 2006; Iovine, 2013). *Salmonella* isolates have been reported to be intrinsically resistant to erythromycin via active efflux, but

naturally susceptible to azithromycin (Gunell *et al.*, 2010). Azithromycin has been suggested as a drug for treating non-typhoidal *Salmonella enterica* infections (Gunell *et al.*, 2010).

Cephalosporins

Cephalosporins are β -lactam antibiotics that inhibit cell wall biosynthesis. In particular, extended-spectrum cephalosporins are used to combat a wide range of bacterial pathogens (Lupo *et al.*, 2013). Since the late eighties, *Salmonella* spp. has shown increased resistance against cephalosporins (Arlet *et al.*, 2006). Liebana *et al.* (2013) recently reviewed the role of enterobacterial isolates, including *Salmonella*, in the production of ESBLs in food and animals. A variety of *Salmonella* serotypes has been associated with the spread of ESBLs in poultry, cattle and pigs. ESBLs confer resistance to a variety of β -lactamases, including penicillins, first-, second-, third, and fourth-generation cephalosporins, and monobactams, but not carbapenems (Boyle *et al.*, 2010; Liebana *et al.*, 2013; Lupo *et al.*, 2013). Nowadays, the most widespread ESBLs belong to the CTX-M family (Lupo *et al.*, 2013). Special attention is also paid to metallo- β -lactamases which could render bacteria resistant to most β -lactam antibiotics, including also carbapenems (Arlet *et al.*, 2006). Although progress has been made in identifying some metallo- β -lactamases inhibitors, no approved drug which targets metallo- β -lactamases, is currently available on the market (Fast and Sutton, 2013). Most ESBL-AmpC-producing strains have been reported to carry additional resistance genes to other commonly-used veterinary drugs. Therefore, generic antimicrobial use is a risk factor for co-resistance (EFSA, 2011; EFSA and CDC, 2011).

7. Alternatives for antibiotics in animal nutrition

Livestock performance and feed efficiency are closely related to the microbial load of the animal gut, the morphological structure of the intestinal wall and the activity of the immune system. In this context, antimicrobial growth promoters (AMGPs) were introduced in intensive animal husbandry (Lallès *et al.*, 2009). Based on increasing concerns on the development of AMR bacteria due to intensive antibiotic use, European Commission decided to ban all commonly used feed antibiotics in 2006 and to limit the therapeutic use of antibiotics, with a notable exception of coccidiostats and histomonostats. This approach was taken as part of the Community Strategy adopted in June 2001 to combat threats to human and animal health posed by antimicrobial resistance in pathogenic microbes. Two main strategies are explored in order to reduce the use of antibiotics in animals. First, the use of substitutes (functional feed ingredients) with similar effects to AMGPs is envisaged and, second, the overall improvement of animal health via improved management practices (Lalles *et al.*, 2009).

Strategy 1: management practices as infection control measures

Improved management practices (MP) face limitations in terms of controlling the infection and indigenous microbiota composition as components of animal health. Many of these limitations are related to diverse management practices, ranging from intensive indoor rearing to extensive, largely outdoor, rearing systems. Europe envisages a strong trend towards improved welfare of farm animals. As a consequence, outdoor 'green' and more sustainable production systems have been increasingly integrated with intensive indoor housing in the pig industry. These measurements may have a dual effect on animal health. On the one hand, outdoor reared animals may harbour a more complex commensal microbiota and therefore they may be naturally more resistant to infections. On the other hand, pigs reared under such conditions face increased contact with potentially pathogenic microbes in the environment, which can then be re-introduced in intensive indoor housing systems. Gebreyes *et al.* (2008) showed that the seroprevalence of *Salmonella* and *Toxoplasma* in pigs reared in outdoor antimicrobial-free pig production system was higher than in pigs from intensive indoor production systems. The right balance between outdoor and indoor rearing systems in terms of impact on complexity of the pig's commensal gut microbiota, gut physiology and immune system development is one of the critical issues.

Another MP consists of the 'all-in-all-out' method of livestock production. Such system replaces the earlier technique of having a constant stream of animals moving through the farm. Instead of having animals with a range of ages, all animals with similar characteristics are designated into a single cohort and are housed together in one shed. They are not allowed to mix with animals from other cohorts so cross-infection between groups is prevented (Cameron, 2000). A study conducted by Namata *et al.* (2009) showed that the 'all-in-all-out' principle was effective in reducing the risk for *Salmonella* infection in broiler chicken flocks.

The 'specific pathogen-free' (SPF) system is another approach to control the health of production animals by preventing the contamination with pathogens in the very beginning of their life, without antibiotic intervention. SPF animals are bred under controlled conditions, such as facilities and are subject to extensive monitoring systems in order to keep the animal stock free of specific pathogens. For instance, a common technique in pig breeding is the development and maintenance of pathogen free pig populations by hysterectomy, hysterotomy or snatch farrowing (Cameron, 2000). This will only be cost-effective for valuable breeding stock, like specialty pigs for niche markets or chicken flocks for the production of vaccines.

Vaccination is another intervention possibility to reduce pathogens in livestock and poultry (Allen *et al.*, 2013; Zoete *et al.*, 2007). Regulation (EC) No. 2160/2003 sets community targets and programmes (e.g. vaccination) for the reduction of prevalence of all *Salmonella* serotypes with public health significance in breeding flocks of *Gallus gallus* (chicken), laying hens, broilers, turkeys, herds of slaughter pigs and breeding herds of pigs (EC, 2003). One of the major drawbacks in all these MPs is the high cost involved. Large Australian and USA farms along with the majority of intensive European farms have implemented the previous mentioned infection control interventions and especially the 'all-in-all-out' system.

Strategy 2: functional feed ingredient use

Certain functional feed ingredients (FFI) are known to have a modulating effect on microbiota composition and pathogen susceptibility in livestock, improving livestock performance, feed efficiency and animal health. These FFI include (in-feed) enzymes, probiotics, prebiotics, organic acids, medium chain fatty acids and plant extracts and etheric oils (De Lange *et al.*, 2010; Lalles *et al.*, 2009). Compounds used within the EU need to be registered and EU Register of feed additives is updated regularly by the Commission (EC, 2003; 2013). Before registration, the EFSA Panel on Additives and Products or Substances in Animal Feed (FEEDAP) assesses the additives and products or substances used in animal feed. The FEEDAP panel provides independent scientific advice on the safety and/or efficacy of additives/ingredients used in animal feed. The panel evaluates their safety and/or efficacy for the target species, the user, the consumer of products of animal origin and the environment.

In-feed enzymes

Nowadays, in-feed enzymes are routinely added to livestock feeds to break down certain components of the feed, including: β -glucans, xylans, proteins, and phytases that may cause digestion problems or act as antinutritional factors (Adeola and Cowieson, 2011). Most commonly used in-feed enzymes, applicable as FFI, are β -glucanase, xylanase, phytase and β -mannanase (Adeola and Cowieson, 2011).

Competitive exclusion and probiotics

Competitive exclusion products have been widely used in Finland and Sweden. The so-called 'Nurmi concept' and competitive exclusion (CE) involves oral administration of intestinal microbiota from healthy *Salmonella*-free adult birds into newly hatched chicks and has been proved effective in numerous laboratory trials (Schneitz, 2005; Schneitz and Hakkinen, 1998). In addition to pathogen control, it has been demonstrated that in field trials CE

treatment enhances the growth and decreases the mortality of birds and improves the feed conversion (Schneitz, 2005).

Probiotics are similar to CE products. They have been reported to improve the overall health of an animal by improving the commensal microbiota balance in its gut (Dankowiakowska *et al.*, 2013; Gaggia *et al.*, 2010). The mechanisms that mediate this effect have not been firmly established. It has been hypothesised that they act via one or more of the followings: (1) reiteration of the competitive exclusion principle: by colonising the gut in large numbers, probiotic microbes exclude pathogens and thus prevent them from causing infection; (2) stimulus for the immune system (immunomodulation): as the immune system is engaged following exposure to probiotic bacteria, pathogens are also detected, following increased surveillance by leukocytes, and thus potential pathogens are eliminated; and (3) strong, positive influence on intestinal metabolic activities, such as increased production of vitamin B12, antimicrobial peptides (e.g. bacteriocins), and short chain fatty acid, such as propionic acid. Other mechanisms have been proposed but remain to be confirmed. Probiotics have been shown to be effective in new-born animals or those that have been treated with antibiotics (Gaggia *et al.*, 2010). Combination of probiotics with other dietary supplements, e.g. prebiotics, has been reported to improve feed conversion ratio in broilers (Bozkurt *et al.*, 2009). The most commonly used probiotic strains applicable as FFI are *Bacillus subtilis*, *Enterococcus faecium*, *Pediococcus acidilactici*, *Lactobacillus* spp. and *Saccharomyces cerevisiae* (EC, 2014).

Prebiotics (and symbiotic)

Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gaggia *et al.*, 2010). As is the case of probiotics essential information concerning the impact of prebiotic on animal health is still incomplete. Thus, the most important future target for prebiotic research will be to demonstrate health-benefit supported by knowledge on the mechanism of action. Combinations of suitable probiotics and prebiotics (also called symbiotic) may prove to be the next tool to reduce the risk of intestinal diseases and disorders in livestock. Most research on prebiotics in production animals has been performed in poultry (Bozkurt, 2009; Gaggia *et al.*, 2010; Vandeplas *et al.*, 2010). Fructo-oligosaccharides and manna-oligosaccharides are known to promote the colonisation of beneficial bacteria in poultry, which in turn improves feed efficiency and reduces disease severity and mortality (Yang *et al.*, 2008). However, few studies have addressed the mechanisms underlying such improvements.

Organic acids

In commercial compound feeds, organic acids have been used for decades for feed preservation. For this reason, most organic acids and their salts are classified as 'feed preservatives' within the EU. Acidifiers can also be used safely and effectively together with other additives. The main mode of action of organic acids is through their antimicrobial effects, the magnitude of which is dependent on the chemical properties of the individual organic acid or its salt. The market for organic acids is expected to continue to grow, especially in regions that ban antimicrobial growth promoters (AMGP), reflecting the industry's move away from antibiotic growth promoters. Northern Europe started to adopt these products before the EU ban of AMGP. Most commonly used acidifiers, applicable as FFI, are benzoic acid, formic acid, propionic acid, lactic acid, citric acid, malic acid, fumaric acid, sorbic acid and all their salts (EC, 2014; Kluge *et al.*, 2006).

Medium chain fatty acids

Medium chain fatty acids (MCFAs) have been considered as effective substitutes for AMGP. In particular, MCFAs with 6 to 12 carbon atoms have been shown to exhibit antibacterial effects. Administration of MCFAs in feed improves livestock performance and feed efficiency (de Lange *et al.*, 2010; Rossi *et al.*, 2010; Zentek *et al.*, 2011). Examples of medium chain fatty acids are caproic acid (C6), caprylic acid (C8), capric acid (C10) and lauric acid (C12). However, none of these MCFAs is authorised as feed additives in EU (but are allowed as feedstuffs (EC, 2013a).

Natural plant extracts and essential oils

Medicinal plants are a rich source of bioactive components (alkaloids, flavonoids, phenols, terpenoids, steroids, essential oils) and over 1000 plants, herbs and essential oils have been shown to exhibit antimicrobial effects (Schelz *et al.*, 2010). Phytochemicals are a group of natural growth promoters used as feed additives, derived from herbs, spices or other plants. There are several phytochemicals on the market. Recently, encapsulation techniques have been applied to enhance the stability of phytochemicals in feed applications. Currently, phytochemical substances are classified as sensory additives according to the EC (2003) and are intended to increase feed aroma and palatability (Maenner *et al.*, 2011). Windisch *et al.* (2008) concluded that a systematic approach towards the efficacy and safety evaluation of phytochemical compounds used as feed additives for pigs and poultry are still missing. However, in the recent years the number of publications on this topic has increased (Maenner *et al.*, 2011). Several studies have evaluated the *in vitro* antimicrobial activities of various essential oils (including clove, rosemary, thyme, tea tree, oregano) against pathogens such as *Listeria monocytogenes*,

Salmonella spp., *Campylobacter* spp., *E. coli* O157:H7, *Shigella dysenteriae*, *Bacillus cereus*, *Staphylococcus aureus* and *Vibrio* spp. (Aslim and Yucel, 2008; Cheng *et al.*, 2014; Dorman and Deans, 2000; Randrianarivelo *et al.*, 2009). The inhibitory activity results from a complex interaction between their different constituents, which may produce additive, synergistic or antagonistic effects, even for substances present at low concentrations (Burt, 2004). Some components of essential oils (carvacol, thymol, cinnamaldehyde) have been shown to reduce the antibiotic resistance of zoonotic pathogen strains like *S. enterica* serotype Typhimurium, *E. coli*, *S. aureus*, and *Streptococcus pyogenes* (Palaniappan and Holley, 2010). Essential oils could be a relevant alternative to antibiotics in shrimp hatchery, since they have been shown to reduce *Vibrio* spp. levels in *Penaeus monodon* (shrimp) larval cultures, similar to erythromycin E antibiotic, (Randrianarivelo *et al.*, 2010). In addition, plant extracts can also act as immuno-stimulants by enhancing both specific and non-specific defence mechanisms of animals, thus increasing their disease resistance. Several herbal immuno-stimulants have been reported to increase the innate and adaptive immune response in fish against bacterial, viral and parasitic diseases (Harikrishnan, 2011). Recently, Niewold (2014) stated that promotion of growth and health in production animals largely depends on the attenuation of postprandial inflammation. Plants and plant extracts are a potential source for anti-inflammatory compounds and could be utilised as alternatives for antibiotic growth promoters. However, the efficacy of the compounds needs to be further verified both in *in vivo* assays and in animal feeding trials.

8. Other perspectives

In Canada, withdrawal of ceftiofur, a cephalosporin, for prophylaxis in chicken hatcheries resulted in the reduction of ceftiofur-resistant *Salmonella* Heidelberg and *E. coli* in human cases and in retail poultry. This is a good example showing why restrictions in the use of clinically important antibiotics should be enforced (Dutil *et al.*, 2010). However, there is an ongoing debate about the role of antibiotic use in animals and the development of drug-resistant bacterial populations in humans (Cox and Ricci, 2008; Marshall and Levy, 2011). In some cases, it has been reported that changes in the prevalence of resistance of *Salmonella* strains did not correlate with changes in the veterinary use of the drug (Threlfall *et al.*, 2006). Instead, for *Salmonella* serotype Enteritidis, foreign travel and consumption of imported foods contaminated with drug-resistant strains were important contributors to the increased prevalence of resistance (Miriagou *et al.*, 2004). In their study on *Salmonella* isolates collected during a ten year period from Danish pigs, Emborg *et al.* (2008) concluded that the use of antimicrobial agents may select multiple resistant clones and this may cause changes in the antimicrobial resistance within a serotype rather than emergence of

resistance within clones. Their results also supported the view that susceptible serotypes only slowly become resistant to antimicrobials. Future developments in non-phenotypic tests, including next-generation sequencing enable early and more sensitive detection of resistance mechanisms and characterisation of persistent strains (Diaz-Sanchez *et al.*, 2013; Lupo *et al.*, 2013).

Sub-clinical *Salmonella* infections or healthy carriage in animals can be common. Bacteria may spread rapidly and easily between animals in the herd or flock without causing any clinical symptoms in the animals. In some cases, animals can become intermittent or persistent carriers (EFSA and CDC, 2014). The high prevalence of ESBL/ampC-producing bacteria in the poultry production system and their association with public health problems is currently one of the most problematic matters in antimicrobial resistance (EFSA, 2011). In the recent years, there has been increased concern for increased number of methicillin resistant *S. aureus* in pork (EFSA and CDC, 2014). Extensive trade and movement of animals can increase the selection and dissemination of antibiotic resistant bacteria and genes (EFSA, 2011). Antibiotic resistance may also be caused by illegal or off-label use of approved products (WHO, 2011b).

9. Conclusions and recommendations

Antibiotic resistance is a global concern. Hence international co-operation, communication and control methods are needed. Prevention of both emergency and spread of antibiotic resistant bacteria is necessary for the control of antibiotic resistant bacteria and genes in the food chain. Implementation of high level farm biosecurity and control on animal trade along with good hygiene in the food chain are important issues in the prevention of spread of food pathogens and zoonotic bacteria, including *Salmonella* and *Campylobacter*. There is a need to reduce the overall antimicrobial burden, especially the use of 3rd and 4th generation cephalosporins should be better controlled as well as other antimicrobials not strictly needed for veterinary purposes. The animal-to-human transmission of antibiotic resistance needs to be further investigated. For instance, we need to understand the role of environmental bacteria as reservoir for antibiotic resistance genes.

Moreover, monitoring and surveillance systems need to be harmonised in order to obtain data sets suitable for risk analysis of AMR spread (Aidara-Kane *et al.*, 2013). The resistance to currently existing antibiotics is increasing dramatically and consumption of antibiotics is expected to increase. Alternative antimicrobials suitable as feed additives are needed. A multitude of phytochemicals are known to have antimicrobial activities, but their current use as feed additives is more focused on the sensory qualities and palatability of the feed. Also, the potential of probiotics and prebiotics to prevent the growth and spread of important

animal pathogens is largely unknown. However, efficacy of the compounds needs to be further verified both in *in vivo* assays and in animal feeding trials. More research is needed to develop synergistic animal feeding and husbandry strategies that would enable to further reduce the need to use antibiotics in the treatment of herds of production animals.

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