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REVIEW

Fluoroquinolone pollution of food, water and soil, and bacterial resistance

Aura Rusu · Gabriel Hancu · Valentina Uivaroşi

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Abstract Fluoroquinolones are a valuable synthetic antibacterial class widely used in the treatment of infectious diseases both in humans and animals. Until recently, it has been thought that bacterial resistance to fluoroquinolones develops very slowly. Nowadays, there are multiple studies that reveal the alarming occurrence of bacterial resistance and there is a high risk of becoming therapeutically useless. The emergence of this phenomenon comes from injudicious usage in therapy, the presence of residues and their metabolites in food of animal origin and also in sewage, compost and domestic waste, which end up in soil and water sources. In the present paper, we reviewed important issues regarding fluoroquinolones impact on the environment in connection with the development of bacterial resistance: (1) the presence of fluoroquinolones as pollutants in soil, surface waters, and food. Fluoroquinolones are persistent with high specificity to interact with soil compared to other antibiotics. Pollution of water sources raises concerns regarding the effects of small concentrations (ng L^{-1}) on human health and also of the environment. The non-therapeutic use in animal farms conducts to food pollution; the cultivated plants could concentrate the fluoroquinolones (over 100 μg L^{-1}); (2) the increase of bacterial resistance to fluoroquinolones occurring with specific mutations in the target enzymes as well by the plasmid-mediated resistance and active efflux of the cell; (3) international regulations of the fluoroquinolone residues in food that are far to encompass all compounds; (4) fluoroquinolones residues analysis with standardized methods should provide limits of detection lower than maximum residue limit values; and (5) trends and perspectives: (a) a wider process of harmonization of regulations; (b) the fluoroquinolones restriction, necessary for low levels of bacterial resistance; (c) the soil and waste water purification methods; (d) the practice of soil planting scheme as an alternative; and (e) an environmental label in order to facilitate the selection of drugs.

Keywords Fluoroquinolones · Bacterial resistance · Environmental pollution · Antibiotic · Occurrence

Introduction

Unreasonable use of antibiotics in the veterinary and human medicine, particularly in the livestock production units, has led to the emergence of the global environmental pollution problem, namely the environmental pollution with antibiotics. The antibiotics used for treatment of various types of infections in humans and animals are disposed unchanged or as metabolites, thereupon through different excretion mechanisms end up into the wastewater and soil. Particular cases are those of the antibiotics that are added directly in the water, in fish farms, leading to high concentrations of antibiotics in both water and lake sediments. On the other hand, many antibiotics are used also in the treatment of infected plants. However, these quantities can be considered as being small in comparison with the amounts of antibiotics used in human and veterinary

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medicine as well as in livestock production (Sarmah et al. 2006; Martinez 2009; Kümmerer 2009). However, in the last decade, antibiotic pollution of the environment through plant cultivation started to be a source of pollution that needs to be taken into account (Li et al. 2008). The pharmaceutical industry, in some countries, is also a major source of environmental pollution with antibiotics, even if legislative aspects are usually well regulated (Larsson et al. 2007).

Fluoroquinolones are synthetic antibacterials widely used in the treatment of infectious diseases both in humans and in animals. Their great therapeutic value and importance are reflected also by the fact that the development of bacterial resistance to these compounds is less common than for other classes of antibiotics (Sharma et al. 2009). The development of antimicrobial resistance to fluoroquinolones has led to alert the international authorities (World Health Organization - WHO, World Organisation for Animal Health—OIE), as it is already known and demonstrated that this phenomenon is present and its incidence is increasing gradually (EMEA/CVMP/SAGAM/184651/2005-CONSULTATION, FDA Veterinary Newsletter 2001). Because the unreasonable use of fluoroquinolones and environmental pollution with these compounds through various sources, the phenomenon of increased bacterial resistance to fluoroquinolones is growing. High morbidity and mortality in critically ill patients are closely related to multidrug-resistant bacteria. The European Centre for Disease Prevention and Control (ECDC) reported in 2009 that 25,000 patients die annually due to installation of bacterial resistance to antibiotics. Also in Europe, antibiotic-resistant bacteria produce huge costs and decrease productivity by at least 1.5 billion euros per year, according to a 2009 European Medicines Agency report (EMEA/576176/2009). In this review, we intend to present a summary of recent data regarding the use of fluoroquinolones in therapy, environmental pollution, and increase of the bacterial resistance phenomenon to this modern group of antibacterials. This paper is intended as a warning addressed to health professionals (physicians and pharmacists), researchers, and decision makers from institutions involved in regulating the use of fluoroquinolones and environmental pollution by pharmaceuticals.

Antibacterial quinolones as pollutants

The antibacterial quinolones represent a class of therapeutically valuable synthetic compounds used in both human and veterinary medicine, which currently are among the most prescribed antimicrobial agents. These compounds, generally called "quinolones", are effective in many types of bacterial infection and therefore are indicated in the treatment of a wide range of local and systemic infections. The history of quinolone antibacterial compounds started with nalidixic acid as compound model and continued with new compounds in order to improve the unsatisfactory tolerance and increase the activity spectrum (Lesher et al. 1962; Ball 2000; Li 2006; Beale 2011). With the introduction of a fluorine atom and a piperazine substituent, this class of compounds has experienced a significant improvement in the therapeutic properties through spectrum activity broadening (including *Pseudomonas aeruginosa* and Gram-positive cocci) and optimization of pharmacokinetic properties. Nowadays, numerous representatives are used both in human (Table 1) and in veterinary (Table 2) medicine. Quinolone derivatives, which share a 6-fluoro substituent, are known under the generic name of "fluoroquinolones", being currently by far the most frequently used antibacterial quinolones (Ball 2000; Anand and Remers 2003; Martinez et al. 2006; Beale 2011).

Nowadays, ciprofloxacin is the most prescribed fluoroquinolones in the world, followed by ofloxacin, levofloxacin, lomefloxacin, norfloxacin, and sparfloxacin. Norfloxacin is commonly used in Europe, but no longer used in the USA (Andreu et al. 2007). Some compounds have been specially developed for veterinary use, a classic example being enrofloxacin, the first veterinary fluoroquinolone. In the USA, six fluoroquinolones are mainly used in veterinary medicine: enrofloxacin, difloxacin, danofloxacin, marbofloxacin, orbifloxacin, and sarafloxacin (Table 3) (Martinez et al. 2006). Among the fluoroquinolones approved to be used in aquaculture is included sarafloxacin (Serrano 2005). Pradofloxacin is a veterinary fluoroquinolone recently approved (2011) in the European Union by the European Medicines Agency (Veraflox–Bayer) (EMA/CVMP/479774/2010). Pradofloxacin is a third-generation fluoroquinolone, approved by the European Medicines Agency for the treatment of infections in dogs and cats and recently approved in the USA but only for the treatment of skin infections in cattle.Structurally, pradofloxacin is the pure SS enantiomer, similar to moxifloxacin (Table 1). Antimicrobial spectrum includes Gram-negative and Gram-positive bacteria, anaerobic bacteria, *Mycoplasma spp.,* and other micro-organisms (*Rickettsia* and *Mycobacterium spp.*) (Lees 2013).

A particular importance represents the microspecies of fluoroquinolones that can be found in the environment. Under various pH conditions, fluoroquinolones can be neutral, cationic, anionic, or zwitterionic and their physicochemical and biological properties may change. Protonation constants of quinolone derivatives can be described by three macroconstantes and four microconstantes (Rusu et al. 2012). Fluoroquinolones are excreted unchanged or can suffer conjugation, oxidation, hydroxylation, dealkylation (e.g. enrofloxacin is de-ethylated to ciprofloxacin), or

Table 1 Antibacterial quinolone derivatives commonly used in human medicine

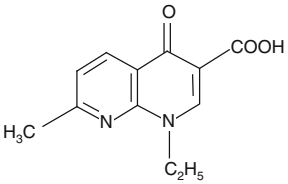
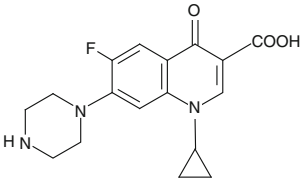
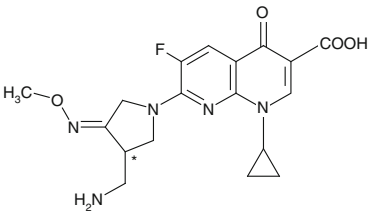
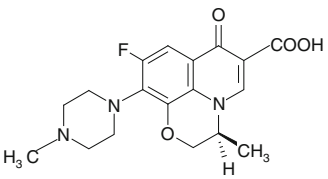
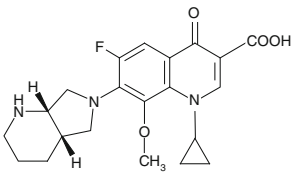
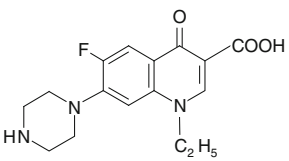
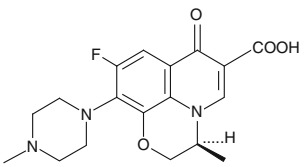
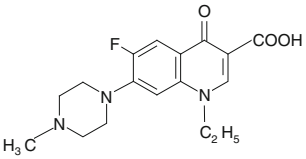
Compounds/chemical structures	Systematic names (IUPAC)
Nalidixic acid 	1-Ethyl-7-methyl-4-oxo-[1,8]naphthyridine-3-carboxylic acid
Ciprofloxacin 	1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro-quinoline-3-carboxylic acid
Gemifloxacin 	7-[(4Z)-3-(Aminomethyl)-4-methoxyimino-pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid
Levofloxacin 	(S)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid
Moxifloxacin 	1-Cyclopropyl-7-[(1S,6S)-2,8-diazabicyclo [4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid
Norfloxacin 	1-Ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid
Ofloxacin 	(RS)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid

Table 1 continued

Compounds/chemical structures	Systematic names (IUPAC)
Pefloxacin 	1-Ethyl-6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid

decarboxylation processes. Significant amounts of compounds (most of fluoroquinolones are not metabolized) and theirs metabolites can reach the soil via urine, faeces, or manure. In a large study regarding evaluation of the excretion masses and environmental occurrence of 50 antibiotics, including fluoroquinolones, in southern China animal farms, 28 antibiotics were detected in the feeds, animal wastes, and environment. Interesting was the fact that ciprofloxacin showed the lowest concentration (0.1 mg kg^{-1}) compared with the highest concentration (183.5 mg kg^{-1} for oxytetracycline). Therefore, a simple calculation of the total excretion masses of antibiotics leads to a result of 3,080 tons per year in China (Zhou et al. 2013). Some studies have been concentrated on the amount of antibiotics in vegetable farmland in southern China, and quinolones had the highest amount (195.3 μg kg^{-1}), which is over than ecotoxic effect trigger value (100 μg kg^{-1}) (Rehman et al. 2013).

The compounds end up as pollutants, mainly in the composition of the slurry sludge as well as in river waters. The sludge is mostly used as fertilizer of the soils for cultivation of various plants. Another possibility for fluoroquinolones pollution is the application of antibacterial treatments in fish farms; as a consequence, the fish became polluted by fluoroquinolones or their metabolites. Drug manufacturers contribute to a lesser extent to fluoroquinolones pollution because nowadays there are strict regulations and control in this area. The pollution potential of unused or expired medicines which are owned by patients cannot be monitored and quantified, but is considered less relevant in terms of pollution. Fluoroquinolones residues in food of animal origin can jeopardize consumer's health, through the emergence of bacterial resistance to these substances (Fig. 1) (Andreu et al. 2007; Sukul and Spiteller 2007; Frade et al. 2014; Manzetti and Ghisi 2014).

The studies published so far do not clarify, however, the fate of these compounds and their metabolites once introduced in the environment. The emergence of antibiotics in soil and water has led to the intensification of the research activity in order to identify possible hazards. Predicted

Table 2 Antibacterial quinolone derivatives commonly used in veterinary medicine

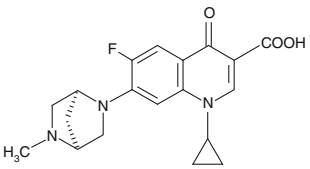
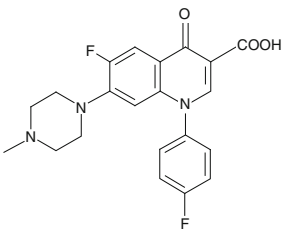
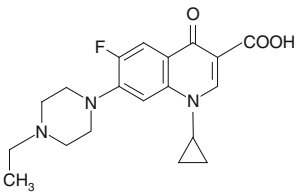
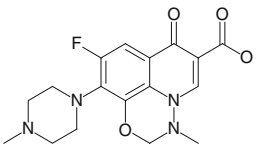
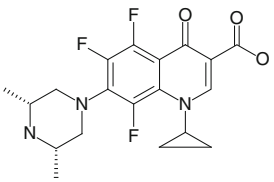
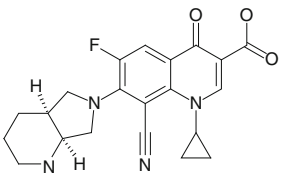
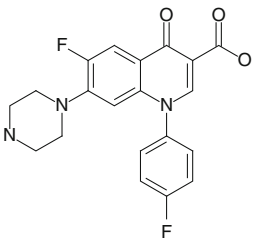
Compounds/chemical structures	Systematic names (IUPAC)
Danofloxacin 	1-Cyclopropyl-6-fluoro-7-[(1S,4S)-3-methyl-3,6-diazabicyclo[2.2.1]heptan-6-yl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid
Difloxacin 	6-Fluoro-1-(4-fluorophenyl)-7-(4-methylpiperazin-1-yl)- 4-oxo-1,4-dihydro-quinoline-3-carboxylic acid
Enrofloxacin 	1-Cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid
Marbofloxacin 	9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyridol(3,2,1-ij)(4,2,1)benzoxadiazin-6 carboxylic acid
Orbifloxacin 	1-Cyclopropyl-7-[(3 s,5r)-3,5-dimethylpiperazin-1-yl]-5,6,8-trifluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid
Pradofloxacin 	8-Cyano-1-cyclopropyl-7-(1S,6S)-2,8-diazabicyclo-(4.3.0)nonan-8-yl)-6-fluoro-4-oxo-1,4-dihydro-3-quinoline carboxylic acid
Sarafloxacin 	6-Fluoro-1-(4-fluorophenyl)-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid

Table 3 Fluoroquinolones elimination pathways on different animal species

Fluoroquinolone	Species	Elimination pathways
Danofloxacin	Cattle	Elimination by urine (90 % unchanged) and faeces (57 % unchanged) in equal amounts
Difloxacin	Dog	Elimination by faeces 80 % (20 % as major metabolites, glucuronide, and desmethyl derivatives)
Enrofloxacin	Dog Cat Cattle	Mostly metabolized in the liver, it is converted to ciprofloxacin and other minor metabolites. Small amounts of ciprofloxacin were determined in the blood of the cats (20 % of metabolites) when compared to dogs and cattle (50 % of the metabolite)
Marbofloxacin	Dog Cat	Less metabolized, mostly eliminated by urine. Small amounts of desmethyl and N-oxide metabolites are present in dogs, pigs, rats, and cows
Orbifloxacin	Dog	After 72 h: Dogs: elimination by urine 28 % (13 % glucuronide compound) and by faeces 15 %;
	Cat	Cats: elimination by urine 45 % (4 % *N*-hydroxylated compound) and by faeces 18 %

Thus, the fluoroquinolones are variable depending on the species metabolised a significant percentage of the administrated amount is eliminated unchanged (Martinez et al. 2006)

environmental concentration (PEC) in different compartments is a widely accepted parameter regarding the risk assessment procedure to antibiotics. PEC value should be compared with the predicted no effect concentration (PNEC). The ratio between PEC and PNEC is the risk coefficient (RQ), which must be <1. If the risk coefficient is $\leq$1.95, the active substance is not considered an environmental hazard. Calculation of the PEC takes in consideration the scenario in which the active substance is excreted 100 % in unchanged state (Sukul and Spiteller 2007).

Similar to other pollutants, photochemical degradation is another pathway for the fluoroquinolones to go through in the environment (Albini and Monti 2003; Prabhakaran et al. 2009; Sturini et al. (2012a, b). The studies on this issue are not numerous, and it is not clear whether the products resulting by photochemical degradation present or no antibacterial activity and represent a potential risk to the environment and human health. By irradiation, chemical changes occur in the structure, but usually quinolone ring remains intact and the antibacterial activity of the compounds is generally high. The presence and persistence of even small quantities of antibacterial agents into the environment may result in micro-organism resistance to

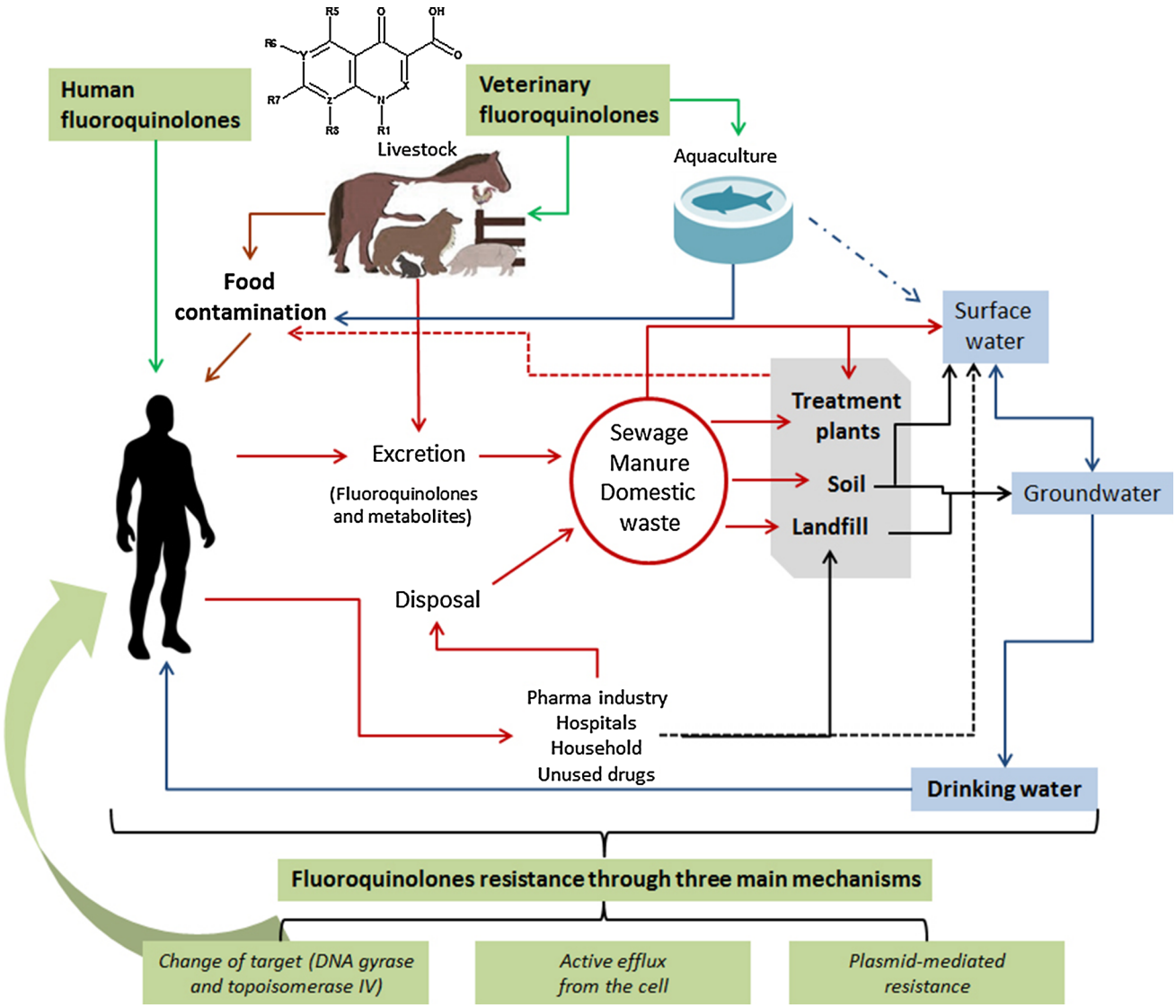


Fig. 1 Pollution with fluoroquinolones and the main mechanisms of fluoroquinolones resistance. The fluoroquinolones and theirs metabolites end up in the sewage, manure, and domestic waste and then in soil and waters. In addition, the presence of fluoroquinolones residues in food of animal origin causes the emergence of bacterial resistance to these compounds by the main mechanism of installation

antibiotics. In the same time, photochemical degradation can be an appropriate route for suppressing the antimicrobial activity of these compounds (Sturini et al. 2012a, b).

Soil pollution with fluoroquinolones

Fluoroquinolones are among the most persistent classes of antibiotics in soil; they remain in the soil for months after fertilization with bio-solid materials, which represent a high risk for the development of bacterial resistance (Ernervik 2011). An illustrative example is Brazil, a country where livestock production is one of the main economic activities and antibiotics are widely used for the treatment of animals. Since there are few data about the appearance, behaviour, and impacts of antibiotics in Brazilian soil, sorption behaviour was assessed for four widely used fluoroquinolones and five sulphonamides in thirteen types of soils with different physical, chemical, and mineralogical properties. Fluoroquinolones sorption phenomenon was much higher in comparison with the sulphonamides one. However, it seems that fluoroquinolones will be not transferred in the soil, even in extreme situations where the soil is rich or poor in organic compounds (Leal et al. 2013). Another recent study on residues from chicken farms and soil samples, also from Brazil, showed that fluoroquinolones have a high specificity for interaction with soils. The high concentrations of

fluoroquinolones demonstrate that chicken production is a potential source of environmental pollution, which so far has been ignored and requires a deeper investigation (Leal et al. 2012).

Antibacterials in general, including here fluoroquinolones, can be found in activated sludge, and active concentrations of the antibiotic charge throughout the year are correlated with the variation of the annual consumption data, being higher in the winter. High concentrations of ciprofloxacin, 426 mg/kg, were determined in urban sewage sludge. High concentrations of norfloxacin, 22 mg/kg, and of ciprofloxacin, 20 mg/kg, were found also in compost. These values represent a warning signal for the use of sewage sludge to make compost and also for using compost as fertilizer for food plants, requiring that fertilizer to be tested for the presence of antibiotics (Gobel et al. 2005; Lillenberg et al. 2010).

Water pollution with fluoroquinolones

Surface water plays an important role in the emergence and spread of antibiotic resistance, so development of strategies to improve water quality became a permanent necessity. Some studies that evaluate different approaches, which can be applied when assessing the potential health risks associated with indirect exposure to pharmaceuticals, metabolites, and degradation products in drinking water, have been already published. Regular medication and also the illicit fluoroquinolones contribute to the excretion of unchanged fluoroquinolones and their metabolites in the domestic wastewater; this phenomenon is higher in the emerging pharmaceutical manufacturing countries due to the high population density. Summarizing with all health care units patients, the wastewaters from pharmaceutical manufacturing countries have in content fluoroquinolones and theirs metabolites and also drug-resistant bacteria (Rehman et al. 2013). For this purpose, for a better understanding of the environment role as a reservoir for antibiotic resistance and to elucidate the link between environmental pollution and the emergence of antibiotic resistance, more data and knowledge are needed. Only an integrated view of these two aspects can provide evidence to assess the risk of the spread antibiotic resistance by surface waters and suggests in this context, solutions to this pressing health problem (Lupo et al. 2012; Webb et al. 2003a, b).

The pharmaceutical industry is a big consumer of variety raw materials and in the same time provides large amounts of residuals waters. Unfortunately, there are a lot of production units that do not function according to environmental regulations and expel the untreated wastewaters to the surface waters and in the sewage networks. At the moment, there are different kinds of studies, which conclude the probability of mixing wastewater with other water resources, the most common cases being in developing countries, where the treatment technology is inadequate or inexistent and the environment regulations are too permissive (Rehman et al. 2013). It is concerning the fact that fluoroquinolones are present in sources of drinking water before treatment in low ng L^{-1} concentrations. After the treatment, ciprofloxacin had an incomplete removal from the water in according with a study realized in North Carolina. There are a lot of questions regarding the unknown health's effects even in very small concentrations (Jones et al. 2001).

An experimental study investigated whether exposure to ciprofloxacin affects the marine environment. Degradation of polycyclic aromatic hydrocarbons by natural bacterial communities is an important function of the ecosystem, being a natural remedy of pollutant sediments. Degradation of pyrene, a parameter of this function, was negatively affected by the presence of ciprofloxacin and showed clear dose-effect dependence (Näslund et al. 2008). The surface waters dissolve antibiotics from industry sources, which are linked to various environmental matrices. On the one hand, binding to the soil particles delays biodegradation, but it is known that the soil removed antibiotics from water through association with its constituent elements. Aluminium and iron oxides may alter these interactions by changing surface load. The adsorption of these types of oxides results in the formation of various types of surface complexes, changing the reactivity of the fluoroquinolones in the soil–water interface. The pH or ionic strength changes in the water or soil can alter fluoroquinolones–soil–water interactions causing disposal of fluoroquinolones in soil (Baquero et al. 2008). Interestingly is that in plants, common receptors to fluoroquinolones were identified, affecting chloroplast replication. Ciprofloxacin affects microbial communities, including those that colonize the leaf ageing material (Brain et al. 2008).

Food pollution with fluoroquinolones

A real threat is to induce the resistance phenomenon through contaminated animal food, including here also the contaminated degradation products. In the production units with high livestock densities, development and spread of infectious diseases are favoured, and therefore, the use of antibiotics for anti-infective treatments or even for the purpose of preventing infections is greatly increased. Worrying is that animal farms use huge amounts of antibiotics (e.g. about 11,200 metric tons in the USA and 8,000 tons in China, annual) for non-therapeutic purposes only to promote growth of cattle, pigs, and poultry. Thus, there are favourable conditions for selection, spread, and persistence of antimicrobial-resistant bacteria. Antimicrobial resistance

poses a risk to humans and animals and should be carefully considered because it reduces the effectiveness of antibiotics in the treatment of infectious diseases both in human and in the veterinary medicine (García-Campaña et al. 2009, EMEA/CVMP/416168/2006-FINAL, Kümmerer 2009, Rehman et al. 2013).

On the other hand, some fluoroquinolones, especially the veterinary compounds (e.g. enrofloxacin), have the property to transfer from soil to cultivated plants such as beans, cucumbers, lettuce, and radish. The concentration of enrofloxacin was over 100 μg L^{-1} in roots, cotyledons, and leaves parts of the plants (Regitano and Leal 2010). Therefore, despite the fact that is considered healthy foods, the vegetables can be a source of fluoroquinolones in very small quantities and repeated. Antibiotic pollution of the environment, including with fluoroquinolones, besides the danger of increasing bacterial resistance is suspected to affect the human health. Thus, there is a big question mark between the possible correlation of population growth weight (obesity) and antibiotic use, directly or indirectly through environmental pollution (Ternak 2005).

Mechanism of action of fluoroquinolones

Being highly effective antibacterial agents, fluoroquinolones are widely used both in human and in the veterinary medicine. Fluoroquinolones are known as "topoisomerase poisons" through poison the catalytic activity of the bacterial DNA gyrase (topoisomerase II) and topoisomerase IV. The role of DNA gyrase is to introduce negative supercoils into DNA and also is in charge of removing the torsional stress that accumulates in front of replication forks and transcription complexes. Mainly, the topoisomerase IV serves to remove knots that accumulate in the bacterial chromosome as a result of fundamental cellular processes and decatenating daughter chromosomes following replication and play a less significant role than gyrase in preserving chromosomal superhelical density and reducing torsional stress. The C3 (with a carboxylic substituent) and C4 (-oxo) positions for the fluoroquinolone drugs structure (Table 1) are essential in the interaction process with the target enzymes. Also regarding the fluoroquinolones—enzymes interactions in recent studies—it was confirmed the presence of a water–metal ion (Mg^{2+}) bridge where the serine and acidic residues act as the anchor points that coordinate the bridge to the enzyme. The human type II topoisomerases are characterised by the absence of the serine and acidic residues and consequently are not capable to interact with fluoroquinolones by this mechanism. Although it was long believed that gyrase is the main target of fluoroquinolones in Gram-negative bacteria and topoisomerase IV in Gram-positive bacteria, there are examples of Gram-positive bacteria in which DNA gyrase is the primary target. Today, it is considered an unresolved issue to assess the contribution of the two enzymes in the mechanism of action of fluoroquinolones and it is necessary to study (Beale 2011; Aldred et al. 2014).

Bacterial resistance to fluoroquinolones and other adverse effects

In general, bacterial resistance to antibiotics appears by two ways. First way is known as a "natural" way, when all the strains of the same bacterial species are resistant to a particular drug. This type of antibiotic resistance is known as intrinsic resistance. The second way is "acquired", when resistant strains are having evolved from susceptible ones by selection after mutation or lateral genetic transfer events. In this particular case, there are susceptible and resistant strains in the same species (González-Candelas et al. 2011). Although initially it was thought that bacterial resistance occurs only exceptionally in this antibiotic class; however, in time, it was demonstrated that it develops especially for the first-generation quinolone derivatives. The fluoroquinolones mechanisms of bacterial resistance installation currently known are the following:

Target-mediated fluoroquinolones resistance (DNA gyrase and topoisomerase IV: change of target)

Resistance to fluoroquinolones is installed most often by specific mutations in the two target enzymes. Gyrase and topoisomerase IV mutations are given by aminoacid substitutions in the corresponding gene; the location is referred to *the quinolone resistance-determining region* (QRDR). This region corresponds to the connection between bacterial DNA and the surface of the enzyme and influences the affinity of the fluoroquinolone to the enzyme—bacterial DNA complex. The most common alteration seen is the substitution of leucine for serine or tryptophan (Sharma et al. 2009). In addition, the recent studies reveal that the most common targeted aminoacids are the serine and the acidic residues involved in the water–metal ion bridge (Aldred et al. 2014).

DNA gyrase is the primary target of mutations in Gram-negative bacteria. Additional mutations in *gyrA* and *parC* lead to a high level of resistance in Gram-negative bacteria. After the discovery of topoisomerase IV, it became clear that not only gyrase is an intracellular target of quinolones. The *parC* mutations are the first, which appear in *Streptococcus pneumoniae* and *Staphylococcus aureus,* and are associated with low levels of resistance. Gram-positive resistance bacterial occurs in stages subsequent to the

accumulation of mutations in the *gyrA* which produce high levels of resistance (Ferrero et al. 1995, Muñoz and De La Campa 1996, Bolon 2011). The *parE* or near the *parC* mutations on the species *Escherichia coli* have been identified as factors in the development of a high level of resistance, a similar phenomenon occurring in *Neisseria gonorrhoeae* species.

However, the situation is different in the case of Gram-positive bacteria. For example, clinically isolated *S. aureus*, which was moderately resistant to ciprofloxacin, contained a mutation in the *parC* (grlA), while the isolates, which were resistant to high concentrations of ciprofloxacin, had an additional mutation in the *gyrA* (Bolon 2011). For the new fluoroquinolones (gatifloxacin, moxifloxacin), which bind equally strongly to both target enzymes, the situation is different. Primary mutations that lead to the development of resistance to the fluoroquinolones occur, especially in *gyrA* and less in the *parC* (Fukuda and Hiramatsu 1999; Pestova et al. 2000). The antiresistance molecular mechanism of the new fluoroquinolones has not yet been elucidated and is therefore a great interest for the study of antiresistance of the new compounds. Available crystal structures of *FQ-topoisomerase* (*type II A*)-*bacterial DNA complexes* explained some resistance determinants. Moreover, biological processes occurring in the bacteria under the pressure of the fluoroquinolones contribute to cell death and the development of bacterial resistance, offering new goals to achieve in the case of design of drugs with improved activity and reduced growth ability resistance (Cheng et al. 2013).

Plasmid-mediated resistance

Initially, due to the specific mechanism of action, it was thought that the resistance mediated by plasmid transfer probably will not occur or will be very rarely observed (a plasmid from *Klebsiella pneumoniae* confers resistance to ciprofloxacin). In 2006, it was identified plasmid-mediated resistance of *E.coli* species resistant to several types of drugs, isolated from dogs in a veterinary hospital in Australia. US Agency Food and Drug Administration (FDA) has identified several bacterial species fluoroquinolone-resistant in the human population: *Campylobacter*, *E. Coli*, and *Salmonella* (Pallo-Zimmerman et al. 2010). Plasmid-mediated resistance is related with three family of genes: *qnr* (encode proteins that decrease the binding of the targeted enzymes to DNA), *aa*(*6′*)-*Ib-cr* (encode a variant of aminoglycoside acetyltransferase that acetylates the piperazinic nitrogen atom without substituent from norfloxacin and ciprofloxacin structure; consequently decreases the activity of the two FQ), and *QqxAB, QepA1,* and *QepA2* involved in efflux pump.

The Qnr proteins belong to the pentapeptide repeat family, a series of tandem 5-amino-acid repeats, and are able of protecting DNA gyrase from fluoroquinolones. Genes for these repeat proteins with sequence similarity to plasmid-borne Qnr proteins have been found on the chromosomes of both Gram-positive and Gram-negative bacteria. In the case of Gram-negative bacteria, it has been revealed a mechanism of resistance caused by the presence of a gene *qnr* (in *Klebsiella pneumoniae* initially highlighted). This gene was located on plasmids integrons (correlated with multiresistant to antibiotics) and acts in parallel with mutations in the gene expression of the target enzyme. *Qnr* plasmid-mediated resistance mechanism is the production of a protein that protects DNA gyrase and topoisomerases, from fluoroquinolones inhibition in *Enterobacteriaceae* species. Therefore, this species of bacteria were the most vulnerable to the rapid development and dissemination of multidrug resistance (Strahilevitz et al. 2009; González-Candelas et al. 2011; Bolon 2011; Aldred et al. 2014).

In a recent study, the level of resistance of *E. coli* to some representative fluoroquinolones (ciprofloxacin, enoxacin, enrofloxacin, lomefloxacin, norfloxacin, and ofloxacin) was evaluated by phenotype and genotype into aquatic environment from the region of Osaka, Japan. The detected amounts of fluoroquinolones were comprised of 0.1–570 ng L^{-1}. Fluoroquinolones-resistant *E. coli* (most with mutations in *gyrA*, *parC,* and *parE* regions) were also found, without correlation between the two data types. This is one of the few studies that report the existence of plasmid-mediated resistance in aquatic systems (*Qnr*S1) and provides basic information about the fluoroquinolones antibacterial resistance profile in the Osaka area (Adachi et al. 2013). Another type of plasmid-mediated resistance is by the acetylation of the piperazinyl substituent in the position C7 (ciprofloxacin, norfloxacin) through an aminoglycoside acetyltransferase, AAC (6′)-Ib-cr, encoded by *aa*(*6′*)-*Ib-cr* gene; this type of mechanism is commonly associated with resistance to extended spectrum β-lactamases (Poirel et al. 2008; Strahilevitz et al. 2009; Bolon 2011).

The last category of genes involved in plasmid-mediated resistance of fluoroquinolones is *oqxAB* and *qepA*. There are identified three proteins such as OqxAB (in animal infections), QepA1, and QepA2 (in human infections) involved in efflux pumps as a transporter. The resistance mechanism was identified to be a multidrug efflux pump, OqxAB, which confers resistance to other agents as well, including chloramphenicol. Recently discovered, the QepA proteins displayed a multiple-resistance profile for fluoroquinolones but also for aminoglycosides and broad-spectrum β-lactams (Yamane et al. 2007; Strahilevitz et al. 2009; Aldred et al. 2014).

Table 4 Mechanisms of bacterial resistance to fluoroquinolones in the aquatic environment

Host	Resistance mechanism	Source
A. punctata	QnrVC4	Waste water effluent
Aeromonas allosaccarophila	QnrS	Lake
Aeromonas punctata, *Aeromonas media*	QnrS2	Lake
Aeromonas spp., *E. coli*	QRDR mutations	River and lake, urban effluent
E. coli	QnrS	River
E. coli	OqxAB efflux	Farm water
Metagenome	QepA efflux	River sediment, water from farm environment
P. aeruginosa	QRDR mutations	Hospital and urban waste water effluent

In different kind of water sources, bacteria were detected that occurred different mechanisms of development resistance to fluoroquinolones: plasmid-mediated resistance (Qnr proteins), development of mutations in the quinolone resistance-determining region (QRDR) and proteins involved in efflux pump, OqxAB, and QepA (Lupo et al. 2012)

Active efflux from the cell

Active efflux from the cell is an attribute of the cell membrane that removes residues and other hazardous substances. Generally, the active efflux pump from the cell is responsible for the low levels of fluoroquinolones resistance compared to the induced resistance by mutations occurring at the target enzyme level. The latter one is given by the mutation of genes encoding porins. This mechanism has been described both in Gram-negative and in Gram-positive bacteria (*E. coli*, *P. aeruginosa,* and even *S. aureus,* which does not have an outer membrane cell itself). It seems that fluoroquinolones with a voluminous substituent in C7 position (e.g. moxifloxacin) are less susceptible to bacterial resistance through this mechanism. Sensitivity on the efflux mechanisms correlated with C7 substituent decreases with high probability in order: ciprofloxacin > levofloxacin > sparfloxacin > moxifloxacin. Thereby, the activity of moxifloxacin against *S. pneumoniae* appears to be also a result of decreased efflux from the bacterial cell. Low-level resistance to fluoroquinolones appears when the expression of porins is downregulated. Also enhanced expression of chromosome-encoded efflux pumps also can conduct to fluoroquinolones resistance. Since there is a short time of survival of the bacteria in the presence of fluoroquinolones, during this time, active efflux pump in the cell encourages the development of mutations in the quinolone resistance-determining region, QRDR (Table 4), consequently developing other forms of resistance (Pestova et al. 2000; Laponogov et al. 2010; Sharma et al. 2009; Bolon 2011; Aldred et al. 2014).

In recent years, data have been published on several species of bacteria resistant to ciprofloxacin (in many intensive care units from the Centres for Disease Control and Prevention, USA), ciprofloxacin-resistant *Neisseria gonorrhoeae* (Centres for Disease Control and Prevention, USA), levofloxacin-resistant *S. pneumoniae* (Asia), with the recommendation that ciprofloxacin should not be used in gonococcal infections; fluoroquinolones should not be used if it is not necessary or it is empirical in treatment of urinary tract infections (Aypak et al. 2009; Sharma et al. 2009; Sweetman 2009). International authorities (including the World Health Organization and World Organisation for Animal Health) are already concerned about the development of antimicrobial resistance in humans and animals. The latest available data show that resistance to fluoroquinolones in some parts of Europe is present and growing. Therefore, the use of fluoroquinolones in the animal food, alongside with antiparasitic or insecticides substances, for other reasons than the treatment of clinically diagnosed disease, should be reconsidered (FDA Veterinarian Newsletter 2001, EMEA/CVMP/SAGAM/184651/2005, Love et al. 2010).

The microbial sensitivity to antibiotics is expressed as minimum inhibitory concentration (MIC). Clinically, relevant resistance to antibiotics is defined in terms of the concentration of the antibiotic that can be safely maintained at a target tissue of a patient without causing excessive adverse side effects. The breakpoint (BP) represents minimum inhibitory concentration threshold (typically between 0.25 and 16 μg/mL), which is antimicrobial concentrations achievable in patients. The breakpoint values are standard cut-offs for defining clinically resistant strains. The minimum inhibitory concentrations of sensitive bacteria usually are comprised between 1 ng mL^{-1} and the breakpoint. The bacteria with minimum inhibitory concentration greater than the breakpoint are considered to be resistant to the antibiotic in question. One way to estimate the development of antibiotic resistance is to compare the minimum effective concentration (MEC) with minimum inhibitory concentration and breakpoint or PEC with minimum inhibitory concentration and breakpoint (Table 5).

However, there is no centralized database to quantify fluoroquinolones use in animals, and information on the development of bacterial resistance is not available in all Member States of the European Union, although the latest reports show that resistance in some parts of Europe fluoroquinolones is present and growing (EMEA/CVMP/SAGAM/184651/2005). In the USA, the Food and Drug Administration reported 13.1 million pounds of antibacterial agents used in animal feed in 2009. Many of these

Table 5 Estimation of bacterial resistance development for some fluoroquinolone representatives

Fluoroquinolones	PEC (ng L^{-1})	MIC (μg L^{-1})	PEC/MIC	Breakpoint (BP) (μg L^{-1})	PEC/BP
Ciprofloxacin	1,908	0.001	1.9079	1	0.0019079
Levofloxacin	2,505	0.001	2.5047	2	0.0012524
Moxifloxacin	245	0.001	0.2446	1	0.0002446

Critical concentrations for producing antibacterial effects were assumed to be correspondent to minimum inhibitory concentrations measured for pathogenic microbes. For estimate potential mixture effects, the ratio between predicted environmental concentration (PEC) and minimum inhibitory concentration (MIC) was summed across fluoroquinolones. The breakpoint values are standard cut-offs for defining clinically resistant strains. (Webb et al. 2003a, b; Kostich and Lazorchak 2008; Kostich et al. 2010; Kostich et al. 2014)

antibacterial agents are used also in human medicine. The use of antibiotics in animal feed without any reason related to health issues involves high-risk public health practice and is already correlated with the development of bacterial resistance in humans. On the other hand, it considerably increases the costs of treatment of infections with antimicrobial-resistant organisms occurring in humans. Exposure of the population to antibiotic-resistant species (*Campylobacter*, *Salmonella*) through practices of meat production industry represents a real danger (Love et al. 2010).

International regulations in force

Based on the presented information, the control of antibiotic residues in food and environmental samples is becoming a necessity. The European Union began to regulate this phenomenon through its institutions. Thus, European Union Council Regulation 2377/90/EEC established warning and control plans for the detection of substances and their residues, potentially toxic to consumers, in live animals or animal products used as food. In this document, the terms residue and maximum residue limits of antibiotics (MRL) are stipulated (COUNCIL REGULATION (EEC) No 2377/90 1990). The USA applies a legislated document by the Food and Drug Administration, updated in 2006, "CVM Guidance for Industry. Guidance No. 3, General Principles for Evaluating the Safety of Compounds Used in Food-Producing Animals" (CVM GFI #3 2006). At international level, the Codex Alimentarius Commission (CAC) adopted *"Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programmes Associated with the Use of Veterinary Drugs in Food Producing Animals", "Maximum Residue Limits for Veterinary Drugs in Food",* and other recommendations on the monitoring of residues, official methods of analysis, principles and guidelines for risk analysis. Unfortunately, only danofloxacin and sarafloxacin as fluoroquinolones representatives are comprised in these regulation of Codex Alimentarius Commission (Table 6) (CAC/GL 71-2009, CAC/MRL 2-2012).

Fluoroquinolones residue analysis

To determine the residues of fluoroquinolones, it is necessary to use sensitive and selective methods because environmental and food matrices are complex and fluoroquinolones concentrations are relatively low. There are no regulations to limit concentrations of antibiotics in environmental samples. Instead, the European Union Council Regulation 2377/90/EEC establishes maximum residue limits in food for seven fluoroquinolones: danofloxacin, difloxacin, enrofloxacin, flumequine, marbofloxacin, oxolinic acid, and sarafloxacin. For example, the maximum residue limit for danofloxacin in chicken muscle should not exceed 200 μg kg^{-1}, a similar value with maximum residue limit from Codex Alimentarius Commission guidelines (CAC/MRL 2-2012) (Table 6). The number of regulated fluoroquinolones is too small having in view that in veterinary medicine and animal food production, many other quinolone derivatives are frequently used. The most used compounds in veterinary medicine are amifloxacin, benofloxacin, ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, marbofloxacin, norfloxacin and norfloxacin nicotinate, ofloxacin, orbifloxacin, and sarafloxacin (Sárközy 2001; Mitchell 2006; Wetzstein et al. 2010).

The pharmaceutical industry is also a source of environmental pollution with fluoroquinolones. A study of wastewater from Patancheru, near Hyderabad, India (area with a large number of medicine factories), reveals unexpectedly high concentrations of ciprofloxacin (28,000–31,000 μg L^{-1}), a level higher than the therapeutic plasma levels. Enrofloxacin (780–900 μg L^{-1}), norfloxacin (390–420 μg L^{-1}), lomefloxacin, enoxacin (150–300 μg L^{-1}), and ofloxacin (150–160 μg L^{-1}) have also been identified in a concentration toxic to plants, diatom, blue/ green algae, and/or other bacteria (Larsson et al. 2007). It is clear that the methods of detecting these compounds should provide limit of detection (LOD) values lower than the regulated maximum residue limit values, and at the same time, must quantifies at real and relevant levels the fluoroquinolones in food and environmental samples, in which they could be present (Andreu et al. 2007, EU COUNCIL REGULATION 2377/90/EEC 1990).

Table 6 Maximum residue limits (MLR) values for danofloxacin and sarafloxacin in according with an acceptable daily intake: 0–20 μg kg^{-1} body weight (danofloxacin) and 0–0.3 μg kg^{-1} body weight (sarafloxacin), stipulated by the Codex Alimentarius Commission (CAC) (CAC/MRL 2-2012)

Species	Tissues	Danofloxacin MLR (μg kg^{-1})	Sarafloxacin MLR (μg kg^{-1})	CAC	Notes
Cattle	Muscle	200	–	24th (2001)	
Cattle	Liver	400	–	24th (2001)	
Cattle	Kidney	400	–	24th (2001)	
Cattle	Fat	100	–	24th (2001)	
Chicken	Muscle	200	10	24th (2001)	
Chicken	Liver	400	80	24th (2001)	
Chicken	Kidney	400	80	24th (2001)	
Chicken	Fat	100	20	24th (2001)	Fat/skin in normal proportion
Pig	Muscle	100	–	24th (2001)	
Pig	Liver	50	–	24th (2001)	
Pig	Kidney	200	–	24th (2001)	
Pig	Fat	100	–	24th (2001)	
Turkey	Muscle	–	10	24th (2001)	
Turkey	Liver	–	80	24th (2001)	
Turkey	Kidney	–	80	24th (2001)	
Turkey	Fat		20	24th (2001)	

The analytical methods used in qualitative and quantitative analysis of fluoroquinolones are diverse: chromatographic methods (thin-layer chromatography, gas chromatography, and liquid chromatography), capillary electrophoresis, spectroscopic methods (ultraviolet and visible absorption spectrometry, nuclear magnetic resonance spectroscopy, mass spectrometry, and spectrofluorimetry), and other methods (Andreu et al. 2007; Blasco et al. 2007; Schneider 2009). An immunochemical analytical method based on enzyme-linked immunosorbent assay (ELISA) technique was developed to detect traces of fluoroquinolones in farm animals intended for food consumption, using animal hair samples. The achieved detection level is 10 μg kg^{-1}, and only 50 mg of animal hair is necessary (Fernández et al. 2014). The European Union has issued Commission Decision 657/2002/EC which regulates methods of analysis which are not standardized, including performance criteria, limits, and conditions that must be fulfilled by these methods. The required validation parameters are selectivity, linearity, accuracy, precision, limit of detection, and quantification (COMMISSION DECISION 2002/657/EC).

Trends and perspectives

Since 2005, World Health Organization classified antibiotics by the importance into three categories based on two criteria:

1. Antimicrobial agent or class used as a single therapy to treat a disease in human and
2. The antimicrobial agent or class used to treat diseases caused by organisms that can be transmitted from non-human sources or diseases caused by organisms that may acquire resistance from non-human sources.

Antibiotics are classified as follows:

A. Extremely important antibiotics—fulfil both criteria, in this category, are included quinolone derivatives (fluoroquinolones and other derivatives), aminoglycosides, carbapenems and other penems, third-generation and fourth-generation cephalosporins, cyclic esters, etc.;
B. Very important antibiotics—fulfil only one of the two criteria; members of this category are aminopenicillins, first- and second-generation cephalosporins, penicillins, etc.;
C. Important antibiotics—do not fulfil any of the two criteria (cyclic polypeptides, nitrofurantoin, nitroimidazoles, etc.)

If the resistance is developed by a member of a class, in general, all other members of this group are affected due to cross-resistance. Antimicrobial resistance poses a threat to human health, whether is attributed to human or veterinary medicine. The first revision of this list occurred in 2007, and since then has been reviewed and updated in 2009 and in 2011 (WHO 2011). An alternative to antibiotic resistance phenomenon to different bacteria is the development of new molecules with antibiotic effect. The European Medicines Agency and the European Centre for Disease Prevention and Control organized a conference to determine pharmaceutical companies to speed up the research of

new antibiotic molecules (Carlet et al. 2012). Even more, World Health Organization has proclaimed 7 April 2011 to be dedicated to the phenomenon of bacterial resistance to antibiotics, which aimed to draw a new alarm signal and to converge all international actions, so that mankind does not be discarded in an era similar to the one before the discovery of the antibiotics. The World Health Organization Department of Medicine provides guidance and strategies for regulating the use of drugs by patients, health professionals, and national authorities. Addressing this issue requires a regulatory framework for the use of antibiotics in animal feed, reducing the need and prudent use of antibiotics in growing animals, surveillance of antibiotics use in animal, surveillance of bacterial resistance to antibiotics in food and feed, support and communication activities in this area, training and capacity building, filling data and information gaps and the need for research. The Food and Drug Administration also has developed guidelines for animal production industry regarding the use of antimicrobial agents (CVM GFI#209 2012). A good veterinary practice must be reflected on a rational use of antimicrobials in according with the Food and Drug Administration directives on judicious use on antimicrobials. The way forward will consider the therapeutic efficiency in balance with lowering the selection of resistant micro-organisms.

In Europe, the institutions that support and supervise the organized use of antibiotics are the European Union Directorate General for Health and Europe Centre for Disease Prevention and Control. European Medicines Agency has primary responsibility for the protection and promotion of human and animal health by Annex 1—International Partnership No. 57, evaluation and supervision of medicines for human and veterinary use. Committee for Medicinal Products for Veterinary Use provides scientific advice and opinions on veterinary medicines when they are related to antibiotics. Committee for Medicinal Products for Veterinary Use is supported by the Scientific Advisory Group on Antimicrobials and considers maintaining the effectiveness of antibiotics and minimizing the development of antibiotic resistance as one of the most important tasks in veterinary medicine.

Veterinary Antimicrobial Decision Support is a platform designed in 1997 by several members of the American College of Veterinary Clinical Pharmacology from several USA colleges of veterinary medicine. This platform was supported by the Food and Drug Administration Centre for Veterinary Medicine since 2001. The continued development of this platform is offered by the support of many major organizations: *Academy of Veterinary Consultants, American Association of Bovine Practitioners, National Cattlemen's Beef Association, American Association of Swine Veterinarians, National Pork Board, American Veterinary Medical Association, Food and Drug Administration Centre for Veterinary Medicine, The United States Pharmacopeial Convention*. Veterinary Antimicrobial Decision Support is a platform developed specifically to provide accessible information, comprehensive guidance in the selection of antimicrobial agents for veterinary surgeons to avoid the emergence of bacterial resistance. This platform is based on the existing literature by providing clear and precise, easy to access information.

Another type of intervention is global awareness that antibiotics generally must be judiciously used, only in situations where they are really necessary both in human and in veterinary medicine. In 2006, Codex Alimentarius Commission takes action in this matter and established the Intergovernmental Task Force on Antimicrobial Resistance. At the fourth meeting (October 2010), the Working Group completed its work to develop guidelines regarding antimicrobial resistance risk analysis (ALINORM 10/33/42 2009; REP11/AMR 2010).

In 2009 was established a working Transatlantic Taskforce for Antimicrobial Resistance Group to promote a correlation of US and European activities and programs related to antimicrobial resistance. Following this event, in 2011, was generated a list of 17 recommendations, but without funds to achieve the set goals and no empowering to achieve the global aspects of the problem. Some developed countries begin to reduce antibiotic consumption, but this effort is not always visible in decrease bacterial resistance (Carlet et al. 2012). A positive example is Australia, a country, which has restricted the use fluoroquinolones to humans through its national subsidy system of drugs and by the legislation; it is not allowed their use in production animals. The fluoroquinolones resistance has emerged in the community and remained at low levels for key pathogens such as *E. coli* (Cheng et al. 2012).

Several regional and international alliances and networks have been developed, which have proposed different types of actions, among which they are as follows Action on Antibiotic Resistance, Alliance for the Prudent Use of Antibiotics, the European Society for Clinical Microbiology and Infectious Diseases, Study Group on Antibiotic Policies, and the Alliance against MDRO (multidrug-resistant organisms). To prevent bacterial resistance to fluoroquinolones are searched treatment methods of residues purification of soil and waste waters. In accordance with these directions, Swiss Federal Institute for Environmental Science and Technology conducted a study which has demonstrated that wastewater treatment resulted in a reduction in the fluoroquinolones mass flow rate of 88–92 %, mainly due to the absorption of the sludge treatment (Golet et al. 2003). The disinfection and wastewater treatment can remove up to 80 % of fluoroquinolones (Baquero et al. 2008). Absorption of pollutants by biomass proved to be one of the effective methods for the

Table 7 Classification of some fluoroquinolones according to their environmental impact by following characteristics: persistence, toxicity, ability to resist removal from or degradation in the aquatic environment, bioaccumulation, and accumulation in adipose tissue of aquatic organisms, and the potential to poison aquatic organisms (Stockholm County Council 2014)

Compound	Administration route	Risk	PBT	Observations
Ciprofloxacin	For systemic use	Moderate	6	*RISK* refers to toxic risk to the aquatic environment; the calculation based on Swedish conditions is given as insignificant, low, moderate, or high. *"Cannot be excl"* means that the manufacturer has stated that the documentary basis for assessment of risk is insufficient. Information about environmental risks can be obtained from www.fass.se. *PBT* refers to: P (Persistence) can assume the value 0 or 3 B (Bioaccumulation) can assume the value 0 or 3 T (Toxicity) can assume the value 0–3
Moxifloxacin	For systemic use	Cannot be excl	6*	
Moxifloxacin	Ophthalmological use	Cannot be excl	6*	
Levofloxacin	For systemic use	Cannot be excl	–	
Levofloxacin	Ophthalmological use	Cannot be excl	–	
Ofloxacin	For systemic use	Insignificant	9*	
Norfloxacin	For systemic use	Cannot be excl	–	

removal of pollutants from wastewater. The use of the cork is an alternative for the removal of ofloxacin in aqueous solution at different pH values. The importance of the survey is that cork is a representative of biomass and ofloxacin (a usual fluoroquinolone) is a representative of antibiotics (Crespo-Alonso et al. 2013).

Although there are few studies on soil planting scheme, it appears that there is a link between them and the level of pollution by antibiotics. A recent pilot project conducted in the largest vegetable-producing area in China introduces geographical-detector models to investigate the relationship between planting patterns and residual fluoroquinolones in soil. The encouraging results show that it is possible to introduce effective and practical measures to alleviate pollution of soils by fluoroquinolones. These measures include the planting models and interactions with the amount of manure. Adjustment of the vegetable cultivation models and application of less than 6 kg/m^2 chicken manure annually could be an effective and flexible approach to diminish fluoroquinolones pollution (Li et al. 2013).

The idea of eco-friendly pharmaceuticals was put in practice in Sweden, where the Stockholm County Council, in according to the 2012–2016 Environmental Programme, had finished the assessment and classification of pharmaceuticals according to their environmental impact. It is consider the following characteristics of drugs: persistence, ability to resist removal from or degradation in the aquatic environment, bioaccumulation, and accumulation in adipose tissue of aquatic organisms, toxicity, and the potential to poison aquatic organisms. The environmental impact of fluoroquinolones is presented in Table 6. Also an environmental label is being introduces in order to facilitate the selection the treatment that is the more environment friendly (Jones et al. 2001; Stockholm County Council 2014).

Animal feed is an important source of antimicrobial resistance, even if it is difficult to quantify, it is thought to be comparable to the use of antibiotics in human medicine. To ensure the effectiveness of antimicrobial agents in the future, it is clear that we must act now. Protection of human health requires immediate development and implementation of risk management strategies by authorities regarding the use of fluoroquinolones, cephalosporins of the third and fourth generation and macrolides in animal production units (WHO 2011; Collignon et al. 2009).

Conclusion

Fluoroquinolones are a group of pharmaceuticals, which can be considered to be a valuable therapeutic tool, at which the bacterial resistance installs harder in comparison the other groups of antibiotics. Therefore, measures regarding environmental pollution with this category of antibacterials are imperative in order to prevent or decrease the occurrence of the bacterial resistance phenomenon. Although annual national and international meetings, workshops, and working groups are organized, and there are numerous reports in the literature and press releases, which were dedicated to environmental pollution by antibiotics, they nevertheless have a limited impact due to the lack of convergence (Table 7).

Reducing environmental pollution by fluoroquinolones imposes global measures and harmonization of legislation. Among the most useful measures include as follows:

- The judicious use of fluoroquinolones in human and veterinary medicine;

- Interdiction of fluoroquinolones for human use in veterinary medicine;
- Quitting preventively (including fluoroquinolones) use of antibiotics in livestock and poultry farms;
- The judicious use of fluoroquinolones in fish farms and the treatment of various crops;
- The treatment of antibiotic contaminated wastewaters (hospitals, farm animals) before being discharged into the environment;
- Estimation of global antibiotics consumption for veterinary use;
- Implementation of consistent and harmonious international legislation.

Since the development microbial agents are in an obvious regression, it is necessary that the existing and valuables therapeutic ones to be used judiciously used at global level. Fluoroquinolones are one of antimicrobials class that deserve to be saved from the installation microbial resistance claws by all possible means.

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