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Review

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Fluoroquinolones in Industrial Poultry Production, Bacterial Resistance and Food Residues: a Review

ABSTRACT

Fluoroquinolones are antimicrobial agents frequently used in poultry production and in human medicine. The use of such substances must comply with safety criteria, including withdrawal periods, doses, and treatment duration, as their misuse and abuse may cause bacterial resistance and the presence of residues in edible tissues. Consequently, the consumption of animal products with fluoroquinolone residues may result in the transmission of resistant bacteria. In addition, if residues are beyond the acceptable levels, fluoroquinolone active metabolites are harmful to human health. This article presents a review on the use of antimicrobials of the fluoroquinolone class in poultry production, focusing on the development of bacterial resistance to these drugs and the presence of their residues in poultry products.

INTRODUCTION

The Brazilian poultry industry is highly developed and has shown extraordinary growth in the last few years (UBABEF, 2011). Brazil is among the largest global producers and the largest exporter of chicken meat. It is also the seventh global egg producer. The quality and competitiveness of Brazilian poultry products are proven by the increase in domestic consumption and by the high exports volumes (Turra, 2012).

The productivity of this industry is supported by the use of modern management and nutritional practices, genetic improvement, modern facilities, and the integrated production system, which was largely stimulated by the partnership between processing companies and farmers, resulting in high quality products and low production costs (Brasil, 2012). This development also raised concerns with the hygiene and health status of chicken products, as demonstrated by the increasing regulations for the production of chicken and egg products. Both the government and the companies heavily invest in programs to ensure the quality of those products (Brasil, 1986; 1994; 1998a). The focus on poultry health was particularly important for the development of the poultry industry.

Pathogen prevention and control are constant challenges. Antimicrobials are frequently used in animal production all over the world for disease treatment and prevention, as well for growth promotion or performance enhancement. There is an increasing concern that the use of these drugs in veterinary medicine, and particularly in animal production, may compromise human health through the selection of resistant bacteria in animals and their transmission to humans through animal foods and dispersion in the environment (Castanon, 2007).

The bacterial resistance to antimicrobials has become a major public interest and scientific issue since the past decade. Although there is



no consensus on the degree of influence of the use of antimicrobials in animals on the emergence and dissemination of bacteria resistant to antimicrobials in humans, experimental evidences and epidemiological and molecular studies point out a relationship between the use of antimicrobials and the emergence of resistant bacterial strains in animals and their dissemination to humans, especially through the food chain.

In addition to the antimicrobial therapy relative risks to the selection pressure leading to the emergence of bacterial resistance, the accumulation of antimicrobial residues in animal products may harm human health, including aplasia of the bone marrow, hypersensitivity reaction, tumor induction, and changes in the normal bacterial flora (WHO, 1995; 2004; Kowalski *et al.*, 2005; WHO, 2009).

This article presents a review on the use of antimicrobials of the fluoroquinolone class in poultry production, focusing on the development of bacterial resistance to these drugs and the presence of their residues in poultry products.

The use of antimicrobials in industrial poultry production

Antibiotic therapy was one of the most important influences on the development of human and veterinary medicine (Finch, 2007), and both its therapeutic and prophylactic use has allowed poultry production to achieve significant productivity improvements (Gonzales *et al.*, 2005).

Antimicrobials started to be used for human therapeutics in the 1930s with sulfonamides. However, during the following years, due to the emergence of resistant microorganisms, resulted in a constant search for more effective therapeutic alternatives in terms of spectrum of action and toxicity (Jackson *et al.*, 1998). Veterinary Medicine started to employ antimicrobials in the 1950s at the same time that advances in human medicine were achieved (EMA, 1999).

In addition of the use of antimicrobials in Veterinary Medicine to prevent and treat diseases, antimicrobials are also used as growth promoters or performance enhancers for livestock. These substances are continuously added in subtherapeutic doses in poultry feeds (Andreatti Filho & Silva, 2005). Their effects were discovered in the 1940s during studies for the isolation and identification of vitamin B12 in fungal cultures, which was considered a growth promoter. In those studies, the dry mycelia of determined fungi, such as *Streptomyces aureofaciens*, contained antimicrobials that worked as potent growth promoters (Jones & Riche, 2003; Castanon, 2007).

The use of antimicrobials as growth promoters, with no need of veterinary prescription, was approved by the US Food and Drug Administration (FDA) in 1951 (Jones; Riche, 2003). In the 1950s and 1960s, European countries approved their own national regulations on the use of antimicrobials in animal feeds.

The inclusion of growth promoters in animal feeds improves feed efficiency, thereby increasing productivity, as well as reduces feed intake up to slaughter, prevents infectious and parasitic pathologies, and decreases mortality rates. However, the modes of action of growth promoters are not fully elucidated, there is a consensus among researchers that they do not have a single, but multiple and complex modes of action (Albuquerque, 2005). In the 1950s and 1960s, penicillins and tetracyclines were used as growth promoters, and in the 1960s, concerns on the emergence of resistant bacterial strains started to be voiced.

In a report to the United Kingdom government, the Joint Committee on the Use of Antimicrobials in Animal Production and Veterinary Medicine acknowledged that the administration of subtherapeutic doses of antimicrobials could represent a risk of human and animal health. In order to prevent such risks, antimicrobial growth promoters should have little or no therapeutic application for humans and animals and should not impair the efficacy of drugs used for therapeutics due to the development of resistant strains. In the 1990s, the debate on the resistance to antimicrobials in the European Union became more intense (EMA, 1999). The European Union then banned the use of avoparcin, virginiamycin, spiramycin, tylosin, and zinc bacitracin in an attempt to minimize the selection of resistant bacteria (Almeida & Palermo-Neto, 2005). In 2006, the use of antimicrobials as growth promoters was banned in the European Union (Palermo-Neto & Renshaw, 2005; Castanon, 2007).

In Brazil, some active principles were banned for the purpose of growth promoters. Among other bans, the Brazilian Ministry of Agriculture (MAPA) issued Order n. 31 banning the use of arsenical and antimonial products (Brasil, 2002). Chloramphenicol and nitrofurans were banned in 2003 by Normative Ruling n. 09 (Brasil, 2003). Normative Ruling n. 17 of 2004 banned substances with thyroidal, androgenic, estrogenic, and gestagenic effects for growth promoting in production animal (Brasil, 2004). Amphenicols, tetracyclines, beta-lactams (benzyl-penicillins and cephalosporins), quinolones, and systemic sulfonamides were banned for that purpose by Normative Ruling n. 26, in 2009



(Brasil, 2009). In 2012, spiramycin and erythromycin were also banned by Normative Ruling n. 14 (Brasil, 2012).

As it is expected that the use of antimicrobials as growth promoters will be increasingly banned and restricted, several research studies have been conducted to search for efficient alternatives, particularly on probiotics, prebiotics, and other means to reduce pathogens in poultry production (Pessoa *et al.*, 2012).

Probiotics are feed supplements containing pure or mixed cultures of live microorganisms that promote beneficial effects in the host by favoring intestinal microbiota balance (Andreatti Filho & Silva, 2005). The mode of action of probiotics is not fully elucidated yet. The main modes of action proposed are competitive exclusion, by which the microorganisms in the probiotic bind to the intestinal mucosa receptors, thereby preventing the adhesion of pathogens to the mucosa; production of antibacterial substances, such as bacteriocins, organic acids, and hydrogen peroxide; competition for nutrients, and stimulation of the host's immune system (Andreatti Filho & Silva, 2005). The efficacy of probiotics is influenced by animal age, type of probiotic, livability of the microorganisms, storage conditions, management conditions and health challenge (Souza *et al.*, 2010).

Prebiotics are substances included individually or associated with probiotics in poultry feeds, which are then called symbiotics. These substances are not affected by digestive enzymes and benefit the host's health by stimulating selectively the growth and metabolism of saprophytic intestinal bacteria (Andreatti Filho & Silva, 2005).

In addition, prebiotics enhance disease resistance, improve nutrient availability, and increase the efficiency of poultry production. The most common prebiotics are fructo-oligosaccharides (FOS), trans-galactooligosaccharides (TOS), inulin, glucooligosaccharides, xylo-oligosaccharides, iso-maltooligosaccharides, soy oligosaccharides, polydextrose, and lactosucrose (Gunguly, 2013).

Other important feed additives used in poultry production are exogenous enzymes that act on feedstuffs, improving their digestion, releasing nutrients and enhancing their absorption, and destroying harmful compounds (Lima, 2005). Plant extracts and essential oils also influence poultry performance. The modes of action of these products are not fully elucidated, but it was shown that plant extracts improve feed digestibility and present antifungal and antimicrobial action, and that essential oils stimulated

the secretion of endogenous enzymes, change the intestinal microflora, and help reducing subclinical infections (Bonato *et al.*, 2008).

In Brazil, in general, there are no statistical data on the use of antimicrobials in animal production. The Department of Health of the state of Paraná conducted a survey in 2004, through the State Program for the Control of Veterinary Drug Residues in Animal Foods (PAMVET), to identify which veterinary drugs were more frequently used for therapeutic purposes in broiler and layer production. In 28 broiler farms, the most frequent antimicrobial groups mentioned were norfloxacin, enrofloxacin, monensin, sulfadiazine+trimethoprim, avilamycin, amoxicillin, clortetracyclin, sulfachlor-peridazin+trimethoprim, maduramicin, nicarbazine, neomycin, tiamulin and tilmicosin (PAMVET, 2005). In 66 layer farms surveyed, the most frequent antimicrobial groups mentioned were quinolones, followed by tetracyclines and quinoloxines. The following drugs most commonly used were enrofloxacin (25.8%), oxitetracyclin (21.5%), olaquinox (15.1%), norfloxacin (9.7%), doxycycline (6.5%), sulfaquinoxaline (6.5%), and bacitracin (6.5%) (Machinski Junior *et al.*, 2005).

The development of fluoroquinolones

The development of quinolones strengthened antimicrobial therapeutics due to its low toxicity and broad spectrum of action. The first quinolone produced was nalidixic acid, which is a naphthyridine. It was discovered by chance by Leshea and coworkers in 1962 during an attempt to synthesize chlorquine, an anti-malarial agent with antibacterial activity. Nalidixic acid presented activity against some Gram-negative aerobes, and was then used for the treatment of urinary infections (Jackson *et al.*, 1998; Silva & Hollenbach, 2010). However, its use was reduced because it tended to induce bacterial resistance and because of the synthesis of new compounds with broader spectrum of action and lower toxicity (Ito, 2005).

The discovery of changes in the chemical structure of quinolones significantly changes their antimicrobial activity, allowing the synthesis of other compounds of this family. The position 1 (Figure 1) of the quinolone molecule is part of the binding complex of the enzyme DNA gyrase and interacts with the hydrophobic site of DNA. Position 2 is very close to the binding site of DNA gyrase (or topoisomerase IV). Positions 3 and 4 are considered critical for binding cleaved or disturbed DNA, and there are no useful substitutions reported to date. The addition of a fluorine molecule in position 6 improved the antimicrobial activity of quinolones compared with the original group, which were then



called fluoroquinolones. Position 7 directly interacts with DNA gyrase. The inclusion of amino-pyrrolidine at this position improves the activity against Gram-positive bacteria, whereas the addition of piperazine increases the activity against Gram-negative bacteria. Changes in positions 5 and 8 change its global steric molecular configuration and target affinity (Peterson, 2001).

Fluoroquinolones were introduced in the 1980s and are fluorinated derivatives of quinolones, i.e., they have fluorine in the position 6 (Figure 1). They also present a ketone in position 4 and a carboxyl in position 3, providing further antibacterial activity. The addition of cyclopropyl, ethyl, or fluorophenyl in position 1 and piperazine in position 7 increased the antimicrobial spectrum of action of fluoroquinolones (Andriole, 2005; Sharma *et al.*, 2009).

The presence of fluorine in position 6 provides greater activity against Gram-positive and Gram-negative bacteria because it enhances the penetration capacity of the antimicrobial drug in the bacterial cell membrane. The first broad-spectrum fluoroquinolone synthesized was norfloxacin, which was launched in 1980. A piperazinyl ring replaces the methyl group at position 7 in this compound, which allowed enhancing the activity of the drug against Gram-negative bacteria, although it did not act against anaerobes (Andriole, 2005).

In ciprofloxacin, the ethyl group of norfloxacin was replaced by a cyclopropyl group, increasing the spectrum of action to include Gram-positive bacteria and not compromising its activity against Gram-negative bacteria (Sharma *et al.*, 2009). The structure of enrofloxacin is similar to ciprofloxacin, with changes in the ethyl group (Ito, 2005).

All these structural changes in the molecule of quinolones provided wider spectrum of action and better diffusion to the tissues, longer half-life, lower toxicity, greater capacity to penetrate the bacterial cell wall, and consequently better activity against Gram-negative bacteria and activity against some Gram-positive species. Their therapeutic indications evolved from urinary infection to applications against many other infections. The last generations present activity against anaerobic bacteria (Sousa *et al.*, 2007; Silva & Hollembach, 2010).

By the end of the 1980s and beginning of the 1990s, fluoroquinolones started to be used in veterinary medicine. Since then, new fluoroquinolone molecules have been licensed, and a large number of different veterinary drugs has been launched in the market (EMA, 2006).

Pharmacodynamics and pharmacokinetics

Fluoroquinolones, as all quinolones, are bactericidal antimicrobials that inhibit the catalytic activity of bacterial DNA gyrase (Topoisomerase II) and Topoisomerase IV, which are essential for the replication and transcription of bacterial DNA (Sharma *et al.*, 2009). DNA gyrase catalyzes reactions in the bacterial DNA double strand chain, allowing its supercoiling, relaxation, separation, and its reintegration during transcription and translation. DNA gyrase consists of two subunits: GyrA (97 kDa), a protein coded by gene *gyrA*, and GyrB (90 kDa), protein coded by gene *gyrB*. Topoisomerase IV also consists of two subunits: ParC (75 kDa) and ParE (70 kDa) (Jacoby, 2005).

After administration, fluoroquinolones are rapidly absorbed, present wide distribution volume and little binding to plasma proteins, are excreted by the urine and bile, and their residues are found in the liver and kidneys (Goetting *et al.*, 2011; Silva & Hollembach, 2010). Enrofloxacin biotransformation includes reactions of N-dealkylation, glucuronide conjugation to the nitrogen in the para position of the piperazinyl ring, oxidation in the ortho position to substituted amine, and the opening of the piperazinyl ring (Vancutsem *et al.*, 1990). Ciprofloxacin is a metabolite of enrofloxacin. Its pharmacokinetics is different and its bioavailability in the bird's body is half or less than a half compared with enrofloxacin (Ito, 2005).

Enrofloxacin has intermediate and variable effect on *Pseudomonas* spp., *Enterococcus* spp., *Clostridium* spp., *Staphylococcus* spp., and *Streptococcus* spp., and good effect on enterobacteria, including *Campylobacter* spp., *Enterobacter* spp., and *Serratia* spp. It is also active against *Chlamydia* spp. and *Mycobacterium* spp. In general, the recommended dose of Enrofloxacin and ciprofloxacin is 10 mg/kg. Because ciprofloxacin is a enrofloxacin metabolite, the amount of ciprofloxacin increases according to the dose and duration of enrofloxacin administration. Enrofloxacin and its analogs are indicated for the treatment of chickens and turkeys against *E.coli*, and of turkeys against *Pasteurella multocida* (Ito, 2005).

Bacterial resistance to fluoroquinolones

International organizations, such as the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organization for Animal Health (OIE), as well as regulating authorities, have expressed their concern with the development of resistance in microorganisms that are pathogenic both for humans and animals,



including zoonotic agents, such as *Campylobacter* spp. and *Salmonella* spp., particularly to some antimicrobial classes, including fluoroquinolones (EMA, 2006).

Bacterial resistance is an inherent side effect of any therapy with antimicrobial agents (Bayer, 1999). In most bacterial species, the resistance to quinolones is caused by mutations in the quinolone targets, which are DNA gyrase and Topoisomerase IV, as well as to mutations that change external membrane permeability, causing the drug to be expelled by active transport (Jacoby, 2005). Resistance mediated by plasmids also occurs, but it is less frequent (Bayer, 1999; Vetting *et al.*, 2011).

Campylobacter is a common bacteria found in the intestinal tract of poultry and its resistance to quinolones is mainly caused by individual mutations in DNA gyrase (gene *gyrA*), and occasionally, in Topoisomerase IV (gene *gyrB*). Despite rare, there are also evidences of resistance by efflux, with consequent cross-resistance to several antimicrobial agents (EFSA, 2009).

Mc Dermott *et al.* (2002) and Van Boven *et al.* (2003) conducted studies on the impact of the use of fluoroquinolones in broilers on the development of resistance in *Campylobacter jejuni*, which is the main agent of bacterial gastroenteritis in the USA. The authors found high minimum inhibitory concentrations (MIC) for enrofloxacin during treatment, and those were maintained after treatment. In addition to bacterial resistance, some studies suggest a possible similarity between fluoroquinolone-resistant *Campylobacter jejuni* clones isolated from poultry samples and those from clinical human samples (Baner, 1985; Endtz *et al.*, 1991; Notario *et al.*, 2011).

Salmonella spp. may develop resistance to fluoroquinolones mediated by chromosomes and by plasmids (PMRQ). The resistance mediated by chromosomes occurs under antimicrobial pressure for specific mutations that result in the substitutions of amino acids within DNA gyrase and Topoisomerase IV at the subunits *gyrA*, *gyrB*, *parC*, or *parE*; reduced expression of porins of the external membrane or lipopolysaccharide (LPS) changes, or superexpression of efflux pumps against several drugs. Mutations in *gyrA*, *gyrB*, *parC*, or *parE* in the regions that are part of the binding site to fluoroquinolones, called "quinolone resistance determining region" (QRDR), change the structure of topoisomerase, preventing fluoroquinolones to bind to this site. Single mutations initially affect only older quinolones, such as nalidixic acid, and additional mutations are required to reduce the susceptibility to fluoroquinolones,

including ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, levofloxacin, and marbofloxacin. These additional mutations lead to the development of "clinical resistance", with MIC higher than 2 mg/L. The resistance to quinolones mediated by plasmids (PMRQ) is mediated by *qnr* genes that code DNA gyrase protecting proteins. However, the basal level of resistance to quinolones offered by *qnr* genes is low. It is clinically important because it increases MIC of *Salmonella* strains resistant to quinolones to clinically relevant levels (EFSA, 2009).

In many countries, fluoroquinolones are first-choice drugs for the treatment of acute gastrointestinal infections caused by *Salmonella* in humans (EMA, 1999), and resistance to this antimicrobial group has often been reported, particularly to nalidixic acid. In a study conducted between 1996 and 2003, 12,252 *Salmonella* isolates were analyzed for antimicrobial susceptibility, and 203 (1.6%) were found to be resistant to nalidixic acid and 14 (7%) were resistant to ciprofloxacin. The resistance to nalidixic acid significantly increased from 0.4% in 1996 to 2.3% in 2003. All isolates resistant to ciprofloxacin presented at least one *gyrA* mutation at the QRDR and did not show any *qnr* or specific *gyrB*, *parC*, or *parE* mutations at this region (Stevenson *et al.*, 2007).

There are reports of bacterial resistance to fluoroquinolones from all over the world. In Latin America, there are high levels of infections caused by multi-resistant *Enterobacteriaceae* species in comparison with other regions of the world. During the last 10 years, urinary infections by *E. coli* and intra-abdominal infections by *E. coli* and *Klebsiella pneumoniae* have shown high resistance rates to trimethoprim/sulfamethoxazole, quinolones, and second-generation cephalosporins, and low resistance levels to aminoglycosides, nitrofurantoin, and fosfomycin (Salles *et al.*, 2013).

In the USA, the *Campylobacter* resistance to antimicrobials increased from 13% in 1997 to almost 25% in 2011. It is estimated that *Campylobacter* causes approximately 1.3 million infections, 13,000 hospitalizations, and 120 deaths each year. Non-typhoid *Salmonella* accounts for about 1.2 million cases, 23,000 hospitalizations, and 450 deaths per year in the United States (CDC, 2013).

A surveillance study on the resistance to fluoroquinolones was conducted between 2007 and 2011 in Canada, under the surveillance program CANWARD. During that period, significant increase was detected in the rate of ciprofloxacin-resistant *E.*



coli, particularly in urine isolates, and a significant reduction in the rate of ciprofloxacin-resistant *S. aureus*. Multi-resistant bacteria, such as *E. coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, except for *Streptococcus pneumoniae*, were frequently resistant to fluoroquinolones (Karłowski *et al.*, 2013).

The 2010 Summary Report on Antimicrobial Resistance of the European Union informed that, among *Salmonella* isolates recovered from human cases, there was a high level of resistance to ampicillin, tetracyclines, and sulfonamides, whereas the resistance to cefotaxime and ciprofloxacin was relatively low. *Campylobacter* isolates from human cases were highly resistant to ampicillin, ciprofloxacin, nalidixic acid, and tetracyclines, but presented low resistance to erythromycin. Among food and animal isolates, the highest incidence of resistance to ciprofloxacin was observed in *Salmonella* from turkeys and chickens (*Gallus gallus*) and from chicken meat. High resistance levels of *E. coli* to ciprofloxacin were observed in chickens and low levels in pigs. In addition, high resistance to ciprofloxacin was reported in *Campylobacter* isolates from chickens, pigs, and cattle, with levels ranging between 37 and 84% (EFSA, 2012).

Antimicrobial residues in poultry products

Another growing concern with the use of antimicrobials in livestock production is the possible presence of antimicrobial residues in animal products and the inherent risks of the consumption of these residues by the population.

Veterinary drugs residues are defined as the fraction of a drug, its metabolites and/or impurities at any edible part of the animal food (Codex Alimentarius, 2011). In human medicine, allergic reactions to drugs are side effects of the therapeutic use of antimicrobials, especially β -lactam drugs. The residues of those compounds in foods may pose risks to humans due to hypersensitivity reactions, as in the case of β -lactams; carcinogenesis associated with chloramphenicol, sulfametazine, and nitrofurans, and selection of bacteria resistant to common antibiotics (Dewdney *et al.*, 1991; Machinski Junior *et al.*, 2005; Goetting *et al.*, 2011).

The Maximum Residue Limit for Veterinary Drugs (MRLVD) is the maximum concentration of residues expressed in mg/kg or μ g/kg that is recommended by the Codex Alimentarius Commission to be legally permitted in or on a food (Codex Alimentarius, 2011). In order to determine MRL, the results of the toxicological analysis and the pharmacokinetics of the

product are considered, as well as the risk or hazard it may pose to human health. The risk is calculated according to the Acceptable Daily Intake (ADI) in mg or μ g/kg of the drug present in the food. The ADI calculation is based on the value of NOEL ("no observed effect level") of a drug, which is the highest level of a chemical compound that does not produce harmful effects.

For the calculation of MRL of antimicrobials used in poultry production, the JECFA (Joint FAO/WHO Expert Committee on Food Additives), a toxicological ADI, derived from toxicity studies, and a microbiological ADI, derived from assays on the possible adverse effects of the drugs on the human gastrointestinal tract and the development of bacterial resistance, are considered. The lowest of those two values are used to determine ADI and for the calculation of the MRL for the antimicrobial drug (Palermo-Neto, 2005).

The JECFA is an international expert scientific committee that meets since 1956 to specifically discuss food additives and it is administered by FAO and WHO. Its work includes the evaluation of contaminants, toxins, and veterinary drug residues in foods, and it is the scientific consulting body of FAO, WHO, member governments, and Codex Alimentarius Commission. It is a reliable consulting source for countries that use this information to formulate their own regulations (WHO, 2013).

Toxicity studies consider that antimicrobial residues that are still microbiologically active in foods may change the intestinal microflora of consumers. However, not all the antimicrobials have a defined MRL. Some are not considered hazardous, and therefore have no MRL, while others have provisional MRL, and others are not allowed to be used in animals (Pereira, 2009).

The European Union has defined MRL for enrofloxacin, which is calculated as the sum of enrofloxacin and ciprofloxacin. In broilers, the defined MRL is 100 μ g/kg for muscle, fat, and skin; 200 μ g/kg for the liver; and 300 μ g/kg for the kidneys. Enrofloxacin and ciprofloxacin cannot be used for poultry that produce eggs for human consumption. The higher MRLs allowed for the liver and the kidneys is consistent with the pharmacokinetics of fluoroquinolones, concentrating higher residue levels in these tissues (European Commission, 2009).

In Brazil, the MRL of enrofloxacin for poultry meat is 100 μ g/kg (Brasil, 2013a). Related to eggs, the reference limit used by the Subprogram of Control Monitoring of Contaminant Residues in eggs effective for 2013 was 10 μ g/kg for enrofloxacin and ciprofloxacin.



Because these drugs are licensed for use in the target species, i.e., layers, but its respective MRL or Maximum Contaminant Content (TMC) has not been established yet by the effective legislation, the Reference Limit for regulatory decision-making is 10 µg/kg or 10 µg/L (Brasil, 2013a).

In Brazil, MRLDV's are established by the Ministry of Health. If these have not been established, the MRLDV's defined by international bodies, such as MERCOSUL, *Codex Alimentarius*, EU directives, and the FDA, are applied. The analytical methods of the National Plan for the Control of Residues and Contaminants (PNCRC) are applied based on the availability of validated analytical methods, particularly those recommended by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF). Brazil has made efforts, through plans and programs of the Ministry of Agriculture (MAPA) and of the National Agency of Health Surveillance (ANVISA) to improve the productivity, quality, and safety of the foods offered to the Brazilian population, and to comply with the health rules of the international food trade recommended by the International Trade Organization (ITO), FAO, OIE, and WHO (Brasil, 1999).

Antimicrobial and anti-parasitic drugs are intensively used in broiler production for disease prevention and treatment, and residues of these products can be found in edible tissues, such as muscles and liver (Goetting *et al.*, 2011). The presence of residues in eggs is a cause of concern because relatively few drugs are indicated for laying hens, despite many are approved for other poultry types (Castanon, 2007). Eggs may contain residues of a wide variety of drugs, which can be detectable days to weeks after the end of the treatment (Goetting *et al.*, 2011). Fluoroquinolone residues, specifically, appear in the eggs 24 hours after the first dose and persist in the yolk and in the albumen for several days after the end of the treatment (Etches, 1998).

In Brazil, several regulations were issued for the control of residues and contaminants in foods (Brasil, 1986; 1995; 1999; 2003b; 2006). MAPA's Normative Ruling n. 07, of March 27, 2013, made public the results of the follow-up of the programs for the control of residues and contaminants of the subprograms of monitoring and surveillance of meats (beef, pork, chicken meat, ostrich meat, and horse meat), milk, eggs, honey, and fish carried out in 2012. There were no reports of non-compliance in 116 analyzed chicken meat samples. The following antimicrobials were analyzed: nalidixic acid, oxolinic acid, ciprofloxacin, difloxacin, enrofloxacin, flumequine, and sarafloxacin.

In eggs, there were 33 analysis for enrofloxacin and ciprofloxacin, in which there has been compliance in 100% of them (Brasil, 2013b).

Studies on fluoroquinolone residues in poultry products

Some studies were conducted, among other objectives, to determine the depletion curve or reduction of fluoroquinolone residue levels in edible tissues and/or eggs after treatment of birds. These studies are particularly important for the improvement of residue monitoring in animal products and for optimization of residue analysis methodologies.

In order to search fluoroquinolone residues in edible chicken tissues, Anadón *et al.* (2001) treated broilers with oral doses of 8 mg ciprofloxacin/kg daily for three days, and then analyzed the liver, kidneys, muscles, and skin+fat. The authors concluded that a withdrawal period of 10 days was sufficient to bring the levels of ciprofloxacin and its metabolites below the MRLDV's established by the EU for enrofloxacin. Jelena *et al.* (2006) analyzed enrofloxacin in the muscle and the liver of broilers orally treated with 10 mg/kg for five days, and determined a withdrawal period of four days to reduce enrofloxacin and ciprofloxacin to acceptable levels.

Gorla *et al.* (1997) analyzed enrofloxacin and ciprofloxacin residues in the eggs of laying hens orally treated with 5 mg/kg twice a day for five days. The authors found that withdrawal periods of six days for enrofloxacin and five days for ciprofloxacin were suitable to avoid violative residues of these antimicrobials in eggs, considering the MRL of 100 µg/kg for muscles, skin, and fat. Lolo *et al.* (2005) treated laying hens with oral enrofloxacin at 12 mg/day for five consecutive days and determined withdrawal periods of eight days for enrofloxacin and seven days for ciprofloxacin. In the study of Cornejo *et al.* (2011), enrofloxacin was orally administered at 10 mg/kg for five days, and a withdrawal period of eight days was sufficient to reduce enrofloxacin and ciprofloxacin levels below the MRL of 100 µg/kg of eggs.

The different proposals of withdrawal periods in those studies may be due to the variability among the methodologies applied to analyze the residues and to the treatment conditions in each experiment.

In Brazil, there are 16 enrofloxacin formulations registered at MAPA and included in the Compendium of Veterinary Products (CPVS) (SINDAN, 2014), and most of them recommend a withdrawal period of seven days before broilers are slaughtered. Related to eggs,



some manufacturers recommend that enrofloxacin should not be used for poultry which eggs are for human consumption, while others do not inform any withdrawal period for laying hens. Therefore, there is no consensus among manufacturers, in general, about the description in the instructions for use related to withdrawal periods, which may allow different interpretation and misuse of these drugs. Consequently, there is a high risk of violating residue levels in edible tissues and in eggs, increasing the risk of consumer exposure to those compounds.

FINAL CONSIDERATIONS

Fluoroquinolones are intensively used in poultry production and have allowed better treatment of several diseases; however, their prudent, ethical, and professional use is essential. The inherent risks of the inadequate use of antimicrobials in poultry production include the induction of bacterial resistance, environmental contamination, and the accumulation of residues in poultry products. The resistance of zoonotic bacteria, such as those belonging to the genera *Salmonella* and *Campylobacter* should be taken into account and prevented, as resistant bacteria or resistance genes may be transferred to humans through the consumption of poultry products. The consumption of poultry products containing high fluoroquinolone residue levels is also a hazard for human health due to their adverse effects, including hypersensitivity reactions and intestinal microflora imbalance, as well as to drug interactions, e.g., they may impair the therapeutic efficacy of other quinolones.

REFERENCES

- Albuquerque R. Antimicrobianos como promotores de crescimento. In: Palermo Neto J, Spinoza HS, Górniak S L, editors. *Farmacologia aplicada á avicultura*. São Paulo: Roca; 2005. Cap. 9, p.149-159.
- Anadón A, Martínez-Larrañaga MR, Iturbe J, Martínez MA, Díaz MJ, Frejo MT, Martínez M. Pharmacokinetics and residues of ciprofloxacin and its metabolites in broiler chickens. *Research in Veterinary Science* 2001;71:101-109.
- Andreatti Filho RL, Silva EN. Pré-bióticos e correlatos na produção avícola. In: Palermo Neto J, Spinoza HS, Górniak SL, editors. *Farmacologia aplicada á avicultura*. São Paulo: Roca; 2005. Cap. 15, p. 225-237.
- Andriole VT. The quinolones: past, present and future. *Clinical Infectious Diseases*. 2005;41:S113-S119.
- Bayer. Resistance. 1999. Available from: <http://www.animalhealth.bayer.com/5168.0.html>
- Bonato MA, Sakomura NK, Piva GH, Barbosa NAA, Mendonça MO, Fernandes JBK. Efeito de acidificantes e extratos vegetais sobre o desempenho e qualidade de ovos de poedeiras comerciais. *Ars Veterinária* 2008;24(3):186-192.
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Portaria nº 51, de 6 de fevereiro de 1986. Instituição do Plano Nacional de Controle de Resíduos Biológicos em Produtos de Origem Animal - PNCRB. *Diário Oficial da República Federativa do Brasil*, Brasília, DF, 07 jul. 1986. Seção 1
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Portaria nº 527, de 15 de agosto de 1995. Adequa o Plano Nacional de Controle de Resíduos Biológicos em Produtos de Origem Animal - PNCRB, de que trata a Portaria Ministerial nº 51, de 06 de fevereiro de 1986. *Diário Oficial da República Federativa do Brasil*, Brasília, DF, 16 ago. 1995. Seção 1
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Portaria nº 46, de 10 de Fevereiro de 1998. Instituir o Sistema de Análise de Perigos e Pontos Críticos de Controle - APPCC a ser implantado, gradativamente, nas indústrias de produtos de origem animal sob o regime do Serviço de Inspeção Federal. *Diário Oficial da República Federativa do Brasil*, Brasília, DF, 16 mar. 1998. Seção 1
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 42 de 20 de dezembro de 1999. Altera o Plano Nacional de Controle de Resíduos em Produtos de Origem Animal - PNCR e os Programas de Controle de Resíduos em Carne - PCRC, Mel – PCRM, Leite – PCRL e Pescado – PCRP. *Diário Oficial da República Federativa do Brasil*, Brasília, DF, 22 dez. 1999. Seção 1, 213p.
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Portaria nº 31, de 29 de janeiro de 2002. Entre outras determinações, proíbe o uso de princípios ativos à base de arsenicais e antimoniais, na fabricação de produtos destinados à alimentação animal, com finalidade de promotores de crescimento ou melhoradores de desempenho animal. *Diário Oficial da República Federativa do Brasil*, Brasília, DF, 5 fev. 2002. Seção 1
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 09, de 27 de junho de 2003. Entre outras determinações, proíbe a fabricação, a manipulação, o fracionamento, a comercialização, a importação e o uso dos princípios ativos cloranfenicol nitrofuranos e os produtos que contenham estes princípios ativos, para uso veterinário e suscetível de emprego na alimentação de todos os animais e insetos. *Diário Oficial da República Federativa do Brasil*, Brasília, DF, 30 jun. 2003ª. Seção 1
- Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução da Diretoria Colegiada nº 253, de 16 de setembro de 2003. Institui o Programa de Análise de Resíduos de Medicamentos Veterinários em Alimentos de Origem Animal (PAMVet). *Diário Oficial da República Federativa do Brasil*, Brasília, DF, 16 set. 2003b. Seção 1
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 11, de 24 de novembro de 2004. Entre outras determinações, proíbe a fabricação, a importação, a comercialização e o uso da substância química denominada Olaquinox, como aditivo promotor de crescimento em animais produtores de alimentos. *Diário Oficial da República Federativa do Brasil*, Brasília, DF, 25 nov. 2004. Seção 1
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 35, de 14 de novembro de 2005. Entre outras determinações, proíbe a fabricação, a importação, a comercialização e o uso de produtos destinados à alimentação animal contendo a substância química denominada Carbadox, como aditivo promotor de crescimento de animais de produção. *Diário Oficial da República Federativa do Brasil*, Brasília, DF, 17 nov. 2005ª. Seção 1
- Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Programa Estadual de Controle de Resíduos de Medicamentos Veterinários em Alimentos de Origem Animal (PAMVET-PR). Levantamento do Uso e Comercialização de Medicamentos Veterinários em Frango de Corte. 23p, 2005b. Available at: <http://www.saude.pr.gov.br/arquivos/File/vigilancia%20sanitaria/Relatorio%20levantamento%20frango.pdf>



- Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Programa de Análise de Resíduos de Medicamentos Veterinários em Alimentos de Origem Animal (PAMVet). Relatório 2004/2005. Monitoramento de Resíduos em Leite Exposto ao Consumo. 46p., 2006. Available at: http://www.anvisa.gov.br/alimentos/pamvet/relat%F3rio_leite_2004-05.pdf
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 26, de 9 de julho de 2009. Aprova o Regulamento Técnico para a fabricação, o controle de qualidade, a comercialização e o emprego de produtos antimicrobianos de uso veterinário, na forma dos Anexos da presente Instrução Normativa. Diário Oficial da República Federativa do Brasil, Brasília, DF, 10 jul. 2009. Seção 1
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 14, de 17 de maio de 2012. Dentre outras determinações, proíbe em todo o território nacional a importação, fabricação e o uso das substâncias antimicrobianas espiramicina e eritromicina com finalidade de aditivo zootécnico melhorador de desempenho na alimentação animal. Diário Oficial da República Federativa do Brasil, Brasília, DF, 18 maio 2012. Seção 1
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 17, de 31 de maio de 2013. Publicar o Subprograma de Monitoramento em Carnes (Bovina, Aves, Suína, Equina, Caprina e Ovina, e de Avestruz), Leite, Pescado, Mel e Ovos para o exercício de 2013, referente ao Plano Nacional de Controle de Resíduos Biológicos em Produtos de Origem Animal – PNCRB, na forma dos Anexos I e II à presente Instrução Normativa. Diário Oficial da República Federativa do Brasil, Brasília, DF, 31 maio 2013. Seção 1, 2013a.
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 07, de 27 de março de 2013. Publicar os resultados do acompanhamento dos Programas de Controle de Resíduos e Contaminantes dos subprogramas de monitoramento e exploratório em Carnes (Bovina, Suína, de Aves, de Avestruz e Equina), em Leite, Ovos, Mel e Pescado do exercício de 2012, na forma dos Anexos à presente Instrução Normativa. Diário Oficial da República Federativa do Brasil, Brasília, DF, 27 mar. 2013. Seção 1, 2013b
- Castanon JIR. History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science* 2007;86(11):2466-2471.
- Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States; 2013. Threat Report, 2013. Available from: <http://www.cdc.gov/drugresistance/threat-report-2013/index.html>
- Codex Alimentarius. Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission. Procedural Manual Twentieth Edition World Health Organization, Food and Agriculture Organization of the United Nations, Roma, 2011. Available from: http://www.codexalimentarius.net/web/procedural_manual.jsp
- Cornejo J, Lapiere L, Iragüen D, Cornejo S, Cassus G, Richter P, San Martin B. Study of enrofloxacin and flumequine residues depletion in eggs of laying hens after oral administration. *Journal of Veterinary Pharmacology and Therapeutics* 2011;35:67-72.
- European Food Safety Authority, European Centre for Disease Prevention and Control, European Medicines Agency, Scientific Committee on Emerging and Newly Identified Health Risks. Joint Opinion on antimicrobial resistance (AMR) focused on zoonotic infections. *EFSA Journal* 2009;7(11):78.
- European Food Safety Authority and European Centre for Disease Prevention and Control; The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in the European Union in 2010. *EFSA Journal* 2012;10(3):233. Available from: <http://www.efsa.europa.eu/efsajournal>
- European Medicines Agency. The European Agency for the Evaluation of Medicinal Products. Antibiotic Resistance in the European Union Associated with Therapeutic Use of Veterinary Medicines. Report and Qualitative Risk Assessment by the Committee for Veterinary Medicinal Products; 1999. 158p. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Report/2009/10/WC500005166.pdf
- European Medicines Agency. Veterinary Medicines and Inspections. Committee for Medicinal Products For Veterinary Use (CVMP). Reflection Paper on the Use of Fluoroquinolones in Food-Producing Animals in the European Union: Development of Resistance and Impact on Human and Animal Health. London; 2006. 23p. Available from: <http://www.emea.eu.int/hums/vet/swp/srantimicrobial.htm>
- Endtz HP, Ruijs GJ, Van Klingeren B, Jansen WH, Van Der Reyden T, Mouton RP. Quinolone resistance in campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. *Journal of Antimicrobial Chemotherapy* 1991;27:199-208.
- Etches RJ. Reproduction in poultry. Wallingford: CAB International; 1998. 318 p.
- European Community. Council Regulation (EC) Nº 2821/98 of 17 December 1998 amending, as regards withdrawal of the authorisation of certain antibiotics, Directive 70/524/EEC concerning additives in feedingstuffs. 1998. 4p. OJ L351.
- European Community. Commission Regulation (EC) Nº 1181/2002 of 1 July 2002 amending Annex I of Council Regulation (EEC) Nº 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Official Journal of the European Communities* 2002; L172:13-20.
- European Community. Regulation (EC) Nº 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. *Official Journal of the European Union* 2003;L268:29-43.
- Finch R. Innovation - drugs and diagnostics. *Journal of Antimicrobial Chemotherapy* 2007;60(suppl1):79 – 82.
- Goetting V, Lee KA, Tell LA. Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: a review of the literature. *Journal of Veterinary Pharmacology and Therapeutics* 2011;34(6):521-556.
- Gonzales E, Café MB, Leandro NSM. Boas práticas no uso de medicamentos pela indústria avícola. In: Palermo-Neto J, Spinosa H, Górniak SL, editors. *Farmacologia aplicada á avicultura*. São Paulo: Roca; 2005. Cap. 18, p. 266-285.
- Gorla N, Chiostrì E, Ugnia L, Weyers A, Giacomelli N, Davicino R, Ovando HG. HPLC residues of enrofloxacin and ciprofloxacin in eggs of laying hens. *International Journal of Antimicrobial Agents* 1997;8:253-256.
- Gunguly S. Nutraceutical and pharmaceutical implication of prebiotics in livestock and poultry feed. Review article. *Bulletin of Pharmaceutical Research* 2013;3(2):71-77.
- Ito NMK, Miyaji CI, Lima EA, Okabayashi S. Antimicrobianos: usos preventivos e curativos em avicultura. In: Palermo Neto J, Spinosa HS, Górniak SL, editors. *Farmacologia aplicada a avicultura*. São Paulo: Roca; 2005. Cap. 8, p. 115-147.
- Jackson LC, Reyes LAM, Cordiés MLH. Quinolonas y terapia antimicrobiana. *Acta Medica* 1998;8(1):58-65.
- Jacoby GA. Mechanisms of Resistance to Quinolones. *Clinical Infectious Diseases* 2005;41(Suppl 2):120 – 126.
- Jelena P, Baltić M, Čupić V, Stefanović D. Residues of enrofloxacin and its main metabolite ciprofloxacin in broiler chickens. *Acta Veterinaria* 2006;56(5-6):497-506.
- Jones FT, Ricke SC. Observations on the history of the development of antibiotics and their use in poultry feeds. *Poultry Science* 2003;82:613-617.



- Karlowski JA, Adam HJ, Desjardins M, Lagacé-Wiens PRS, Hoban DJ, Zhanel GG. Changes in fluoroquinolone resistance over 5 years (CANWARD 2007–11) in bacterial pathogens isolated in Canadian hospitals. *Journal of Antimicrobial Chemotherapy* 2013;68(suppl1):i39-i46.
- Kowalski TJ, Henry M J, Zlabek JA. Furazolidone-Induced Pulmonary Hypersensitivity. *The Annals of Pharmacotherapy* 2005;39:377-379.
- Lima FR. Aditivos zootécnicos: Enzimas. In: Palermo Neto J, Spinosa HS, Górniak SL, editors. *Farmacologia aplicada á avicultura*. São Paulo: Roca; 2005. Cap.16, p.239-248.
- Lolo M, Pedreira S, Fente C, Vázquez BI, Franco CM, Cepeda A. Study of Enrofloxacin Depletion in the Eggs of Laying Hens Using Diphasic Dialysis Extraction/Purification and Determinative HPLC-MS Analysis. *Journal of Agricultural and Food Chemistry* 2005;53: 2849-2852.
- Machinski Junior M, Benini A, Netto DP, Nunes MP, Vedovello Filho D, Benatto A, Scucato ES, Machado E, Belmonte IL, Alberton M, Lopes MO, Bosquiroli SL. Medicamentos veterinários utilizados na avicultura de postura no Estado do Paraná. *Curitiba: PAMvet-PR*; 2005. 24p.
- Mc Dermott PF, Bodies SM, English LL, White DG, Walker RD, Zhao S, Simjee S, Wagner DD. Ciprofloxacin resistance in *Campylobacter jejuni* evolves rapidly in chickens treated with fluoroquinolones. *The Journal of Infectious Diseases* 2010;837-840.
- Notario R, Borda N, Gambandé T, Bermejo J, Ponessa A, Toledo V. Quinolone resistant *Campylobacter jejuni* strains isolated from humans and from poultry. *Medicina* 2011;71(4):331-335.
- Palermo-Neto J. Resíduos de medicamentos veterinários em carne de frango e ovos. In: Palermo Neto J, Spinosa HS, Górniak SL, editors. *Farmacologia aplicada á avicultura*. São Paulo: Roca; 2005. Cap. 19, p. 287-302.
- Pereira AMPT. Determinação de Resíduos de Fluoroquinolonas em Amostras de Tecido Muscular de Frangos e Respectivo Impacto na Saúde Humana [dissertação]. Coimbra (PRT): Faculdade de Medicina da Universidade de Coimbra; 2009. 129p.
- Pessoa GBS, Tavernari FC, Vieira RA, Albino LFT. Novos conceitos em nutrição de aves. *Revista Brasileira de Saúde e Produção Animal* 2012;13(3):755-774.
- Peterson, L. R. Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity. *Clinical Infectious Diseases* 2001;33 (Suppl 3):S180-S186.
- Sharma PC, Jain A, Jain S. Fluoroquinolone antibacterials: a review on chemistry, microbiology and therapeutic prospects. *Acta Poloniae Pharmaceutica - Drug Research*, 2009;66(6):587-604.
- Silva JMB, Hollembach CB. Fluoroquinolonas X resistência bacteriana na medicina veterinária. *Arquivos do Instituto Biológico de São Paulo* 2011;77(2):363-369.
- Sindicado Nacional da Indústria de Produtos para Saúde Animal. *Compêndio de produtos veterinários* [cited 2014 jan]. Available from: <http://www.cpv.com.br/cpv/pesquisar.aspx>.
- Souza LFA, Araújo DN, Astolphi JLL, Dias LBM, Ambiel AC, Santos LS, Carmo AJ, Silva P. CG. Probiótico e antibiótico como promotores de crescimento para frangos de corte. *Colloquium Agrariae* 2010; 6(2):33-39.
- Stevenson JE, Gay K, Barrett TJ, Medalla F, Chiller TM, Angulo FJ. Increase in nalidixic acid resistance among Non-Typhi *Salmonella enterica* isolates in the United States from 1996 to 2003. *Antimicrobial Agents and Chemotherapy* 2007;51(1):195-197.
- Turra F. Mensagem do Presidente Executivo. Relatório Anual 2012. São Paulo: UBABEF; 2012. Available from: <http://www.abef.com.br/ubabef/exibenoticiababef.php?notcodigo=3293>
- União Brasileira de Avicultura. Notícia de 20 de dezembro de 2011. Available from: <http://www.abef.com.br/ubabef/exibenoticiababef.php?notcodigo=3113>.
- Van Boven M, Veldman KT, De Jong CMM, Mevius DJ. Rapid selection of quinolone resistance in *Campylobacter jejuni* but not in *Escherichia coli* in individually housed broilers. *Journal of Antimicrobial Chemotherapy* 2003;52:719-723.
- Vancutsem PM, Babish JG, Schwark WS. The fluoroquinolone antibiotics: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. *Cornell Vet* 1990;80:173-186.
- Vetting MW, Hedge SS, Wang M, Jacoby GA, Hooper DC, Blanchard DC. Structure of QnrB1, a Plasmid-mediated Fluoroquinolone Resistance Factor. *The Journal of Biological Chemistry* 2011;286(28):25265-25273.
- World Health Organization. Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives Evaluation of Certain Veterinary Drug Residues in food [WHO Technical Report Series 851]. Geneva: Who Library Cataloguing in Publication Data; 1995. 42p.
- World Health Organization. Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives Evaluation of certain veterinary drug residues in food [WHO Technical Report Series 954]. Geneva: Who Library Cataloguing in Publication Data; 2009. 135p.
- World Health Organization. About the Joint FAO/WHO Expert Committee on Food Additives. 2013. 4p. Available from: <http://www.who.int/foodsafety/chem/jecfa/about/en/index.html>