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RESISTANCE TO FLUOROQUINOLONES IN *ESCHERICHIA COLI* FROM PIGS

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Abstract

The resistance of *Escherichia coli* (*E. coli*) to fluoroquinolones has become a serious issue on large pig farms worldwide, since these antimicrobial agents are widely used in the control of various diseases such as neonatal diarrhea, post weaning diarrhea, the edema disease as well as others. Being a frequent inhabitant of the digestive tract, *E.coli* is often exposed to antimicrobial agents, which are used to treat various infections. Uncontrolled application of fluoroquinolones has led to the emergence of resistant pathogenic strains as well as commensals. The spread of resistant strains is mostly found in animal and human food production chains, which are potentially huge threat for the general population. The resistance to fluoroquinolones may very often be combined with the resistance to other classes of antibiotics. Therefore, the use of fluoroquinolones for treating uncomplicated infections in pigs must be under strict control or completely banned. In this paper, we compared the results from available literature addressing the prevalence of antimicrobial resistance to fluoroquinolones in *E. coli* strains both worldwide and in countries from the nearby region.

Key words: Escherichia coli, resistance, fluoroquinolones, pigs

REZISTENCIJA *ESCHERICHIA COLI* NA FLUOROHINOLONE KOD SVINJA

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Kratak sadržaj

Rezistencija *Escherichia coli* prema fluorohinolonima postala je ozbiljan problem na velikim farmama svinja u svetu jer se ovi preparati koriste za suzbijanje različitih bolesti, kao što su neonatalna dijareja (neonatal diarrhea), dijareja odlučene prasadi (postweaning diarrhea), edemska bolest (edema disease) i druge. Kao redovan stanovnik digestivnog trakta *E. coli* je često izložena antimikrobnim agenasima koji se koriste u tretmanu različitih infekcija. Nekontrolisana terapija fluorohinolonima dovela je do pojave rezistencije patogenih sojeva, ali i komensala. Širenje rezistentnih sojeva najviše je zastupljeno u lancima proizvodnje hrane za ljude i životinje što predstavlja veliku opasnost po čovečanstvo. Veoma često rezistencija na fluorohinolone može biti udružena sa rezistencijom na ostale klase antibiotika. Upotreba fluorohinolona u tretmanu nekomplikovanih infekcija u svinjarstvu mora biti strogo kontrolisana, ili potpuno zabranjena. U ovom radu upoređeni su rezultati iz dostupne literature o prevalenciji antimikrobne rezistencije na fluorohinolone kod sojeva *Escherichia coli* u svetu i državama iz neposrednog okruženja.

Ključne reči: Escherichia coli, rezistencija, fluorohinoloni, svinje

INTRODUCTION

Intensive pig farming sometimes requires the use of antimicrobial agents for the therapy of various bacterial infections. If the antimicrobial treatment begins without the prior bacteriological examination of the diseased pigs, and if the use of antibiotics is excessive, it may lead to resistance in certain strains of bacteria (Henderiksen et al., 2008). The widespread use of antimicrobial agents has caused the mobilization of bacterial specific mechanisms of resistance in order to survive in the environment (Velhner et al., 2010). In addition to the pathogenic bacteria, commensal microorganisms may also become resistant to antibiotics by creating the possibility for horizontal spread of the resistance genes (Schiearch et al., 2009). Meat and meat products produced for consumption from animals infected with bacteria resistant to antibiotics pre-

sent the major threat to public health (Thorsteinsdottir et al., 2008). In addition to this, multidrug resistant microorganisms have a tendency to expand intercontinentally, which means that some of the bacteria have a unique phenotype and genotype, and may be discovered by using molecular typing methods. The detection of resistance genes and specific mutations on targeted genes could be useful for the epidemiological analysis (Velhner et al., 2010). For example, dihydrofolate reductase genes such as *dfrA1* are more frequent in *E. coli* isolates from Europe while the *dfrA17* and *dfrA12* are more prevalent in Korea and Australia (Blahna et al., 2006). The sequence type ST131 of the *E. coli* resistant to fluoroquinolones and the extended spectrum beta lactam drugs with the CTX-M15 gene is widespread around the world causing urinary tract and bloodstream infections (Petty et al., 2014).

E. coli is a Gram negative bacteria, which belongs to the *Enterobacteriaceae* family and can cause several illnesses in humans and animals. *E.coli* is normally present in the microbiota of the digestive tract in humans and animals, where it is exposed to the influence of various antimicrobial agents, which are used for either therapy or prevention. Consequently, *E. coli* has developed numerous mechanisms, thanks to which it can withstand their influence, where the most important mechanism of resistance is the one towards the fluoroquinolones, due to their intensive and uncontrollable use (Yue et al., 2008). Fluoroquinolones are antimicrobial agents used for the therapy in both humans and animals. Their mode of action is to inhibit topoisomerase enzymes. The resistance develops due to the point mutations on genes which encode the topoisomerase (Hopkins et al., 2005).

MUTATIONS ON THE TOPOISOMERASE GENES

Mutations that cause the development of resistance to fluoroquinolones are located on a protein segment called the quinolone resistance-determining region (QRDR). The *gyrA* and *gyrB* genes encode the gyrase A enzyme. The QRDR is located between amino acids Ala67-Gln106 on the *gyrA* and Asp426-Lys447 on the *gyrB* gene. Single or double mutants are often found on codons 83 and/or 87. The *parC* and *parE* genes encoding the enzyme topoisomerase IV, are the secondary target for quinolone in Gram-negative bacteria. In isolates which demonstrate increased resistance to fluoroquinolones, mutations on all four genes may be found (Hopkins et al., 2005). Resistance to fluoroquinolones can often result in a cross resistance to other antibiotics such as β-lactams, tetracyclines, chloramphenicol and others. Consequently, the health of people and animals may be seriously endangered, if the use of antimicrobial agents should be needed for treatment (Hopkins at al., 2005).

THE SITUATION WORLDWIDE

The *E coli* resistance to fluoroquinolones has become a serious issue on pig farms across the world, since this drug is used for treating several diseases such as neonatal diarrhea, post weaning diarrhea, the edema disease and others. In order to reduce the economical losses caused by these diseases, fluoroquinolones are used in veterinary medicine, which has resulted in the acquisition of the resistance of pathogenic strains of *E coli* and of commensals (Huang et al., 2014). Table 1 shows the licensed quinolones and fluoroquinolones used in livestock production in various regions in the world.

Table 1. Quinolones and fluoroquinolones most commonly used in the livestock industry in various regions around the world (adapted from Webber and Piddock 2001).

Regions	Quinolone type
Europe	Enrofloxacin Flumequine Marbofloxacin Danofloxacin
USA	Enrofloxacin
Japan	Enrofloxacin Danofloxacin Orbifloxacin Difloxacin Ofloxacin Oxolinic acid
Asia	Enrofloxacin Danofloxacin Ciprofloxacin
Latin America	Enrofloxacin Ciprofloxacin Danofloxacin Norfloxacin Flumequine
Canada	None
Australia	None

The most recent research has shown that in China, the *E coli* resistance to fluoroquinolones in farm animals, especially pigs, prevails in comparison

to other countries. High level of resistance to these microbial agents arose as a result of the application of prophylactic antimicrobial therapy during the period of seven days. Because of this established situation, Huan et al. (2014) investigated the effect of ciprofloxacin administered in feed on the dynamics of acquiring the resistance of *E coli* strains isolated from pig feces of the Landrace, Duroc and Yorkshire breed. According to these results, the minimal inhibitory concentration (MIC) for ciprofloxacin in the experimental group, after three days of application, increased from ≥0.5mg/L to ≥8mg/L, while it was stable in the control group ≥1mg/L as well as in the experimental group before the application. After the sixteenth day of application, the MIC for the experimental group was ≥128mg/L. Using the PCR method, point mutations were detected on the *gyrA* and *parC* genes.

According to the research results obtained by the Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM), in *E. coli* (n=358) strains derived from pigs, the resistance to fluoroquinolones (enrofloxacin and ofloxacin) was not established. In most of the isolates (n=267), the MIC ≥0.05mg/L to enrofloxacin was noticed, whereas MIC value for ofloxacin was ≥0.1mg/L (n=230) (Kijima-Tanaka et al., 2003).

A study that examined antimicrobial resistance of zoonotic enteric bacteria *Campylobacter* spp., *E. coli* and *Enterococci* spp. isolated from pig meat and carcasses was conducted in two regions in Australia (New South Wales and South Australia). The resistance to ciprofloxacin has not been established in isolated *E. coli* strains (Hart et al., 2004).

During the year 2004, resistance to ciprofloxacin was not established in *E. coli* strains isolated from feces on 20 pig farms in Canada (in the Alberta state). In the total number of the *E. coli* isolates (n=1439), which were included in this study, the MIC to ciprofloxacin was ≥0.0015mg/L in 1426 of the isolates. The highest MIC of ≥0.5mg/L was observed in one of the examined *E. coli* isolates (Rosengren at al., 2008).

THE SITUATION IN EUROPE

The resistance to fluoroquinolones is mostly observed in multidrug resistant bacteria. Consequently, the therapy for animals and humans is compromised, so caution when using antimicrobial drugs is strongly advised. In some European countries, the use of fluoroquinolones is banned in the animal husbandry.

The legislation in Denmark from 2002 restricts the use of fluoroquinolones in animal husbandry, unless for treating infections, where pathogens are resistant to all the other antimicrobials. According to data from Hammerum

et al. (2007), the consumption of fluoroquinolones in 2001 was around 114 kg. After implementation of this law, the use of fluoroquinolones was reduced to 18 kg in 2005. With the 2005 legislation, the use of fluoroquinolones and cephalosporin's was completely prohibited in livestock production, with special emphasis on pigs, because pig production shares 82% of the total animal production in Denmark. The implementation of this law makes Denmark the only country in Europe where the use of fluoroquinolones (ciprofloxacin, enrofloxacin, difloxacin, and marbofloxacin) is banned in the livestock industry, which was reported by Hendriksen et al. (2008).

In the period 2002-2004, Hendriksen et al. (2008) conducted a study aimed at continuous monitoring of antimicrobial resistance of pathogenic bacteria and commensals, where 12 EU Member States were involved. This was the first report on bacterial resistance to antimicrobial agents in pigs, which also confirmed the resistance of *E. coli* (isolated from pig feces) towards fluoroquniolones (ciprofloxacin and enrofloxacin). While during these three years there was no evidence of *E. coli* resistance to fluoroquinolones in Denmark, Portugal had the highest average prevalence of resistance being 48.0% for the period 2002-2004. Spain is just behind Portugal with an average prevalence of resistance of 14.7%, while in other countries the prevalence of resistance was in the range from 1.3% to 6.5%. Data for the Netherlands was not available, while Lithuania, Norway and Switzerland are lacking evidence for all three years (Table 2).

Table 2: The prevalence of the resistance to ciprofloxacin and enrofloxacin for *E. coli* isolated from diseased pigs in different European countries during 2002-2004., expressed in % (adapted from Hendriksen at al., 2008).

The prevalence of resistance of *E. coli* in some European countries												
Year	B	DK	E	FIN	F	LV	NL	N	P	ES	S	CH
2002	3.3	0.0	8.0	4.0	4.7	-	-	0.0	76.0*	15.0	7.0	-
2003	1.1	0.0	2.0	1.5	6.2	22.0	-	-	38.0*	14.9	0.0	1.3
2004	2.9	0.0	2.0	0.0	5.5	-	-	0.0	30.0*	14.2	6.0	0.0
Average	2.43	0.0	4.0	1.83	5.46	22.0	-	0.0	48.0	14.7	6.5	1.3

B Belgium DK Denmark E England and Wales, FIN Finland, F-France, LV-Lithuania, NL-Netherlands, Norway N-P-Portugal, EC-Spain, Sweden S-CH-Switzerland; *enrofloxacin

According to data from the European Food Safety Authority (EFSA) from 2013, significant decrease of the prevalence of resistance to ciprofloxacin was established in *E. coli* strains isolated from pigs in Holland. While in 2009 the prevalence of the resistance was around 10%, it was not found in any of the 289 *E. coli* strains examined in 2013.

The results obtained by Guerra et al. (2003) revealed that 61% of the analyzed *E. coli* isolates (n = 42) originating from cattle, pigs and poultry in Germany demonstrated no resistance to ciprofloxacin, i.e., the MIC was in the range from ≥0.012mg/L to ≥2mg/L. In 39% of the isolates, the resistance to ciprofloxacin (MIC ≥4mg / L) and the presence of a double mutant on the *gyrA* and *parC* was determined.

The use of antibiotics in the UK on annual basis amounts to 440-480 tons. The half of this amount belongs to tetracyclines, while the other half is shared between sulfonamides, aminoglycosides, β-lactams, macrolides and fluoroquinolones. The prevalence of antimicrobial resistance is different for various domestic animals. The highest resistance is established for tetracyclines used in pig production. There is still no official data on the prevalence of resistance to ciprofloxacin for *E. coli*, isolated from pigs. However from total 2480 of the isolates 0.6% was resistant to nalidixic acid (Enne et al., 2008).

Testing of the resistance to ciprofloxacin and enrofloxacin in Iceland was a part of the research of Thorsteinsdottir et al. (2008) conducted from October 2005 to May 2007. The research objectives was to evaluate the frequency of resistance occurrence and the genetic similarity of *E. coli* strains isolated from healthy pigs and broilers, pig and broiler meat from slaughterhouses, and human isolates. The resistance prevalence is shown in table 3.

Table 3. The prevalence of ciprofloxacin and enrofloxacin resistance of *E. coli* isolated from pigs and pig meat in Iceland expressed in % (adapted from Thorsteinsdottir et al., 2008).

	The source of *E. coli*	
Antibiotic	feces n=109	meat n=50
Ciprofloxacin	14 (12.8)	4 (8.0)
Enrofloxacin	13 (11.9)	3 (6.0)

According to the data available from the literature, the resistance to fluoroquinolones of *E. coli* isolated from the feces of healthy and infected pigs in Europe is higher than in other countries worldwide.

THE SITUATION IN THE REGION

Enrofloxacin and flumequin are the most frequently used quinolones in the livestock industry in Serbia and countries in the region. There is no precise data about the prevalence of the *E. coli* resistance towards quinolones and fluoroquinolones in the surrounding region, except for Croatia and Hungary.

The research conducted by Habrun et al. (2011) in the Republic of Croatia demonstrated an increase in the prevalence of the *E. coli* resistance to fluoroquinolones. In 1990, the resistance was not established, while in 1996 it increased to 7.5%. During the years 1997 and 1998, the prevalence of enrofloxacin-resistance reached 29.7% . The testing conducted on eight pig farms (2005-2007) on 256 *E. coli* isolates isolated from organs of dead piglets with clinical signs of diarrhea revealed an increase of 39% in the resistance prevalence, also towards enrofloxacin, while 11% of the isolates showed intermediate sensitivity. The authors concluded that the increase in the prevalence of the resistance resulted from an excessive use of fluoroquinolones (Habrun et al. 2010).

According to EFSA data from 2013, in Hungary, the prevalence of resistance to ciprofloxacin of *E. coli* isolated from pigs (n = 152) was 9.2%. Hungary is the only country in our region, which uses ciprofloxacin in the livestock industry.

CONCLUSION

Fluoroquinolones are one of the most important classes of antimicrobial agents used for treatment of various infections in swine production. Their uncontrolled use in veterinary medicine may lead to the development of antimicrobial resistance of zoonotic pathogens. Meat and meat products from such animals may present a major reservoir for resistant strains and the source of infection for humans. The transmission of resistant bacterial strains through food and between humans and animals presents a major threat for public health. The use of fluoroquinolones should be limited when treating less dangerous infections or completely prohibited in the livestock industry. Despite numerous studies, the effects of different amino acid substitutions in topoisomerase genes are still not fully understood. In order to overcome the problem of resistance, all antimicrobial agents must be used with caution, especially fluoroquinolones, and the code of practice for issuing prescription for antibiotics through must be respected.

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