THE SITUATION OF ANTIMICROBIAL RESISTANCE OF ENTERIC BACTERIA ISOLATED FROM ANIMAL ORIGIN TO QUINOLONES AND FLUOROQUINOLONES

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Summary. The aim of this study was to determine minimal inhibitory concentration (MIC) values of *Escherichia coli* and *Salmonella enterica* as the most important bacteria family *Enterobacteriaceae* and to evaluate their clinical and epidemiological resistance to quinolones and fluoroquinolones. One hundred and thirty seven strains of *Escherichia coli* and 75 strains of *Salmonella enterica* from different species of animals from different farms (cattle, pigs and poultry) were tested for susceptibility. Nalidixic acid and ciprofloxacin were selected as representatives of quinolones and fluoroquinolones respectively. Results showed that total clinical resistance of *E. coli* isolated from clinical material of animals to nalidixic acid was 44.5%, to ciprofloxacin – and 34.4%. In addition, 22.0% of total *E. coli* were highly resistant to nalidixic acid with MIC value of 256 mg/L and 12.0% of *E. coli* MIC values to ciprofloxacin were also high – 8 mg/L. *S. enterica* demonstrated frequent resistance to nalidixic acid (41.3%) however only 5.3% were resistant to ciprofloxacin with lowest breakpoint value – 0.5 mg/L. *E. coli* isolates from poultry showed to be more frequent resistant to quinolones and fluoroquinolones. Cattle isolates had the lowest frequency of resistance.

Epidemiological susceptibility was counted according to cut-off values (EUCAST). Results showed that 53.0% of *E. coli* had MIC values higher than epidemiological cut-off values to nalidixic acid and 34.0% – to ciprofloxacin. 41.0% of *Salmonella* were epidemiologically resistant to both nalidixic acid and ciprofloxacin and that fact may demonstrate increasing resistance of *Salmonella* to fluoroquinolones.

Keywords: nalidixic acid, ciprofloxacin, *Salmonella*, *Escherichia coli*, clinical resistance, epidemiological resistance.

IŠ GYVŪNŲ IŠSKIRTŲ ENTEROBAKTERIJŲ ANTIMIKROBINIS ATSPARUMAS CHINOLONAMS IR FLUOROCHINOLONAMS

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Santrauka. Darbo tikslas – nustatyti plačiausiai paplitusių ir turinčių svarbiausią klinikinę reikšmę *Enterobacteriaceae* šeimos atstovų *E. coli* ir *Salmonella enterica* MSK (minimali slopinamoji koncentracija) reikšmes nalidikso rūgščiai ir ciprofloksacinui – chinolonų ir fluorochinolonų klasės atstovams – bei įvertinti jų klinikinį ir epidemiologinį atsparumą. Ištirta 137 *E. coli* ir 75 salmonelių padermės, išskirtos skirtingų šalies regionų, galvijų, kiaulių ir vištų fermose. Nustatyta, kad klinikinis *E. coli* atsparumas siekė 44,5 proc. nalidikso rūgščiai ir 34,4 proc. ciprofloksacinui. 22 proc. *E. coli* pasižymėjo ypač dideliu atsparumu nalidikso rūgščiai (MSK 256 mg/L), o 12 proc. – ciprofloksacinui (MSK 8 mg/L). Dažniausiai atsparumu pasižymėjo vištų izoliatai, o rečiausiai – iš galvijų išskirtos *E. coli*. Salmonelės išsiskyrė dažnu klinikiniu atsparumu nalidikso rūgščiai (41,3 proc.), tačiau ciprofloksacinui atsparios salmonelės sudarė tik 5,3 proc., o slopinamoji ciprofloksacino koncentracija buvo žemiausia, nuo kurios padermė laikoma atsparia (0,5 mg/L). Nepaisant to, epidemiologinio jautrumo rodikliai parodė, kad 41 proc. salmonelių ciprofloksacino MSK reikšmės yra palyginti didelės ir viršija natūralias rūšiai būdingas jautrumo ribas. Tai gali rodyti besivystantį salmonelių atsparumą fluorochinolonams.

Raktažodžiai: nalidikso rūgštis, ciprofloksacinas, salmonelės, *Escherichia coli*, klinikinis atsparumas, epidemiologinis atsparumas.

Introduction. Quinolones and fluoroquinolones (newest quinolones) represent a class of antimicrobial agents which is most important in the treatment of severe and invasive infections in humans and animals and are therefore of special interest for public and animal health. Fluoroquinolones were introduced for veterinary use in different countries around the world during the late 80'ties and the beginning of the 90'ties. This introduction and subsequent use has been followed by the emergence of antimicrobial resistance in bacteria of food-producing animals and subsequently spread of resistant zoonotic bacteria to humans (Engberg et al., 2001). The first reported study is from The Netherlands, where water medication with the fluoroquinolone enrofloxacin in the poultry production was followed by an emergence of fluoroquinolone resistant *Campylobacter* strains among both poultry and humans (Endtz et al., 1991). Similar trends have been observed for several *Salmonella* serovars. In Germany, an increase in the incidence of strains of *Salmonella* that are resistant to nalidixic acid was observed after the licensing of enrofloxacin (Malorny et al., 1999). Recent studies in Lithuania showed that resistance among *E. coli* isolated from different domestic animals was 14.7% to ciprofloxacin 22.1% to norfloxacin and 38.2% to nalidixic acid (Ružauskas et al., 2007-2). However, previous studies were based only on qualitative testing.

Fluoroquinolones are very potent antimicrobial agents and active against a wide range of pathogenic organisms and well distributed in the body after administration. This class of antimicrobial agents has a therapeutic effect on most infections in different organs or tissues. Although it is rare that fluoroquinolones are the only available agent for treatment of a specific infectious disease, fluoroquinolones are important alternative medicinal products for a veterinarian to have as option for treatment. Fluoroquinolones have an unique mechanism of action not related to conventional antimicrobial agents, and therefore their efficacy should be retained as long as possible. The (fluoro)quinolones act by inhibition of the activity of the DNA gyrase and in most bacterial species resistance is due to mutations in the gyrase or topoisomerase genes. In *Enterobacteriaceae* resistance to fluoroquinolones is most commonly acquired by mutations in two steps. One mutation in the *gyrA* gene mediates full resistance to first generation quinolones such as nalidixic acid and flumequine and reduced susceptibility to fluoroquinolones. A second mutation in either *gyrA* or *gyrB* genes mediates "full resistance" to fluoroquinolones (Aarestrup et al., 2003). Testing on antimicrobial resistance could be based on qualitative or quantitative methods. Quantitative method is more informative as appropriate inhibition concentrations of antimicrobial are determined. In this case the breakpoint Minimal inhibitory concentration (MIC) is the threshold above which a particular pathogen is unlikely to respond to the specified antimicrobial agent. Breakpoints are used mostly for clinical purpose. A breakpoint for epidemiological purposes (epidemiological cut-off value) takes into account the MIC distribution pattern determined for bacteria populations. It enables identification of two or more populations that can be differentiated by the presence or absence of resistance factors. The wild-type (WT) "susceptible" subpopulation has the MIC profile before any resistance has developed or has been acquired, and its distribution can be differentiated from the "resistant" subpopulation. This epidemiological breakpoint is defined as: epidemiological cut-off value for the WT distribution (also referred to as microbiological breakpoint) and is not necessarily associated with therapy failure (Bywater et al., 2006).

The aim of this study was to determine MIC values of *Escherichia coli* and *Salmonella enterica* as the most common and important pathogenic bacteria family *Enterobacteriaceae* isolated in different animal farms in Lithuania and to evaluate their clinical and epidemiological resistance to quinolones and fluoroquinolones.

Materials and methods. Investigations were performed using field isolates of the most important bacteria of the family *Enterobacteriaceae* – *Escherichia coli* and *Salmonella enterica*. Strains were isolated from herds of cattle, pigs and poultry all over the country from the clinical samples in 2007. The main principle of taking samples was that to obtain herd-representative sample i.e. one isolate from one herd of the same species of bacteria was taken for investigations. The geographical distribution of the herds was wide. Transport media with cotton swabs (Transwab) were used for collecting of material – pathological and clinical samples of diseased animals. McConkey Agar (Oxoid) and TBX Agar (Biolife) were used for isolation of *Escherichia coli*. XLD Agar (Oxoid) together with preenrichment and enrichment media (Bufferied Peptone Water and Rappaport Vassiliadis Broth) were used for isolation of *Salmonella enterica*. Identification was performed using RapID (Remel) identification system together with computer programme ERIC (Remel). MIC (minimal inhibitory concentration) of antimicrobial agents was determined using Sensititre plates (TREK Diagnostic Systems) with different concentrations of antimicrobial agents. Nalidixic acid was used as a representative agent of quinolones and ciprofloxacin – as fluoroquinolones. Preparation of suspensions and inoculations together with reading results was done according to the manufacturer instructions. Interpretation of the results was based on breakpoints and epidemiological-cut-off values using EUCAST database. *E. coli* and *S. enterica* clinical breakpoints to nalidixic acid was counted as ≤ 16 mg/L, to ciprofloxacin – ≤0,5 mg/L. Epidemiological cut-value for *E. coli* and *S. enterica* to nalidixic acid was counted as ≤ 16 mg/L, to ciprofloxacin – 0,03 mg/L for *E. coli* and 0,06 mg/L for *S. enterica*.

Statistical analysis was performed using software Prism 3 (Graphpad, Inc., USA).

Results. Fifty eight *E. coli* isolates from cattle, 44 – from pigs and 35 from poultry (total 137 isolates) were selected and tested for antimicrobial resistance to nalidixic acid and ciprofloxacin (Fig. 1 and 2).

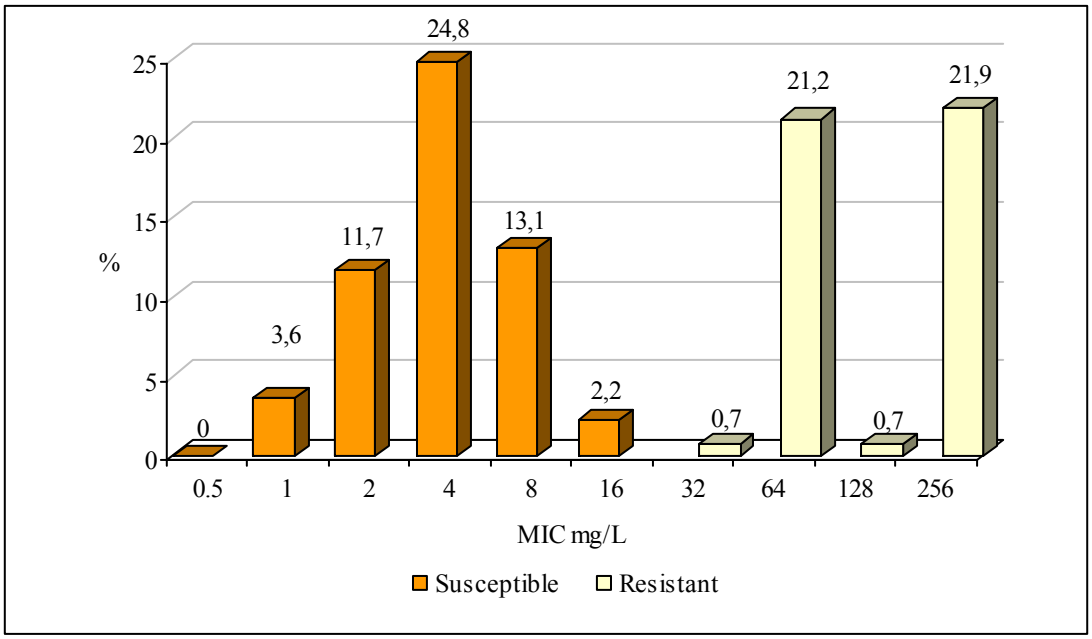

Fig. 1. **Nalidixic acid MIC values to total *Escherichia coli* isolates** (n=137)

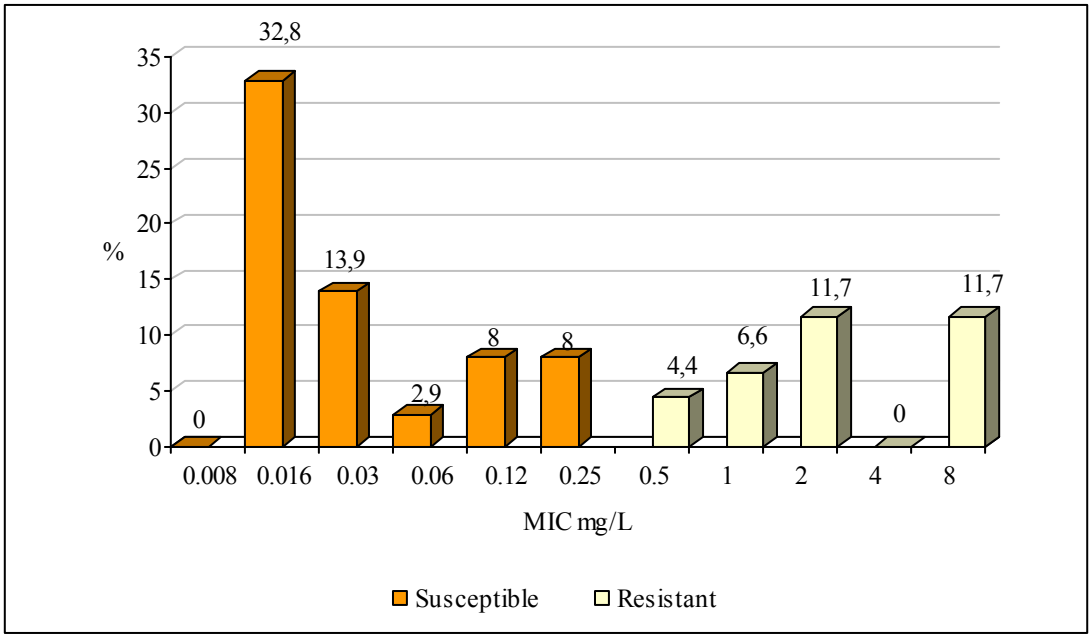

Fig. 2. **Ciprofloxacin MIC values to total *Escherichia coli* isolates** (n=137)

As could be seen from Fig. 1. the biggest number of *E. coli* isolates had MIC range 8 mg/L to nalidixic acid. However even 21.2% and 21.9% had high MIC ranges – 64 mg/L and 256 mg/L respectively. Intermediate MIC range – 128 mg/L had only 0,7 % of isolates. Total clinical resistance of *E. coli* to nalidixic acid was 44.5%. Statistically (in MIC range/percents) the results in "Susceptible" group indicate the 9.233 mean (M), the 9.272 standard deviation (SD), the 3.785 standard error mean (SEM) and the -0.498 – 18.965 in 95% of confidence calculation with lower and upper marks (95% CI). In the "Resistant" group the results were 11.125 (M), the 12.039 (SD), the 6.021(SEM) and the -8.032 – 30.282 (95% CI). The M of difference between "Susceptible" and "Resistant" groups was 1.892, the SD – 12.039, SEM – 6.020 and 95% CI – (-20.254 – 18.054). The statistical investigation of linear and rank correlation between percents of susceptible and resistant groups according MIC range show that the correlation coefficient (r) was 0.456 with rank correlation (S p.r) 0.632, the p value inside groups was 0.785 and 0.588, between groups – 0.271 ($p>0.05$, not significant).

As could be seen from Fig 2. the biggest number of *E.*

coli strains were susceptible to ciprofloxacin at MIC range of 0.016 mg/L. Almost 12.0% of isolates had MIC ranges at 4 mg/L and 8 mg/L. As in the case of resistance to nalidixic acid there were no intermediate strains between those two ranges. Total number of clinical resistance of *E. coli* to ciprofloxacin was 34.4%. The results (in MIC range/percents) in "Susceptible" group indicate the 10.933 mean (M), the 11.732 (SD), the 4.790 (SEM) and the -1.381 – 23.248 (95% CI). In the "Resistant" group the results were 6.881 (M), the 5.001 (SD), the 2.236 (SEM) and the -0.672 – 13.088 (95% CI). The M of difference between "Susceptible" and "Resistant" groups was 4.640, the SD – 12.501, SEM – 5.591 and 95% CI – (-10.879 – 20.159). The linear and rank correlation between percents of susceptible and resistant groups according MIC range show that the correlation coefficient (r) was 0.293 with rank correlation (S p.r) 0.564, the p inside groups was 0.315 and 0.202, between groups – 0.453 (p>0.05, not significant).

75 isolates of *Salmonella enterica* were selected for MIC determination. The results are demonstrated in Fig. 3 and 4.

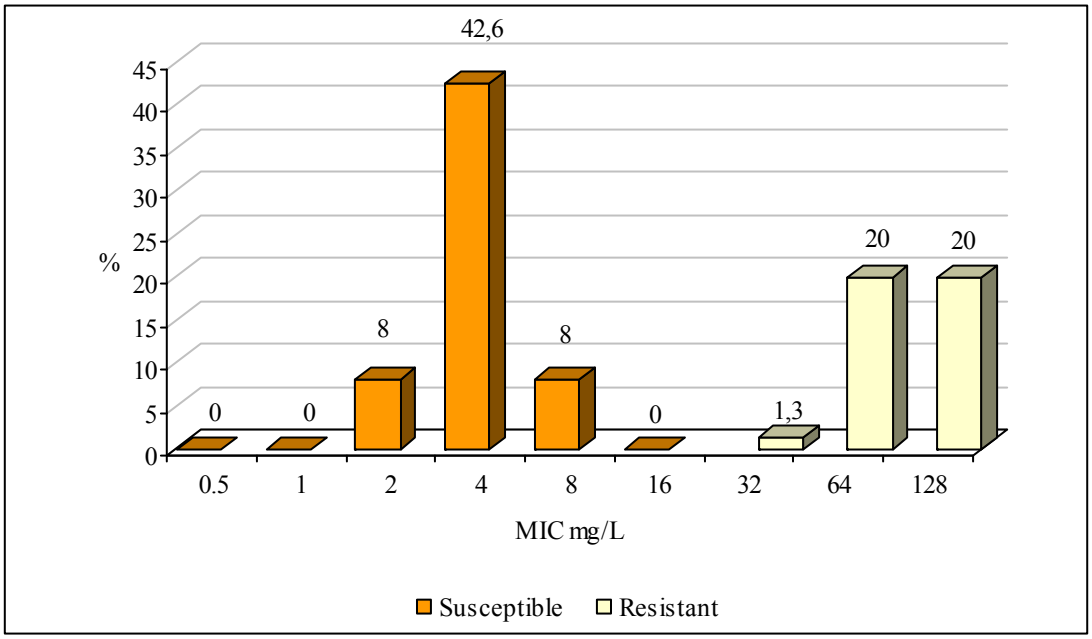

Fig. 3. **Nalidixic acid MIC values and clinical resistance of *Salmonella enterica* isolates** (n=75)

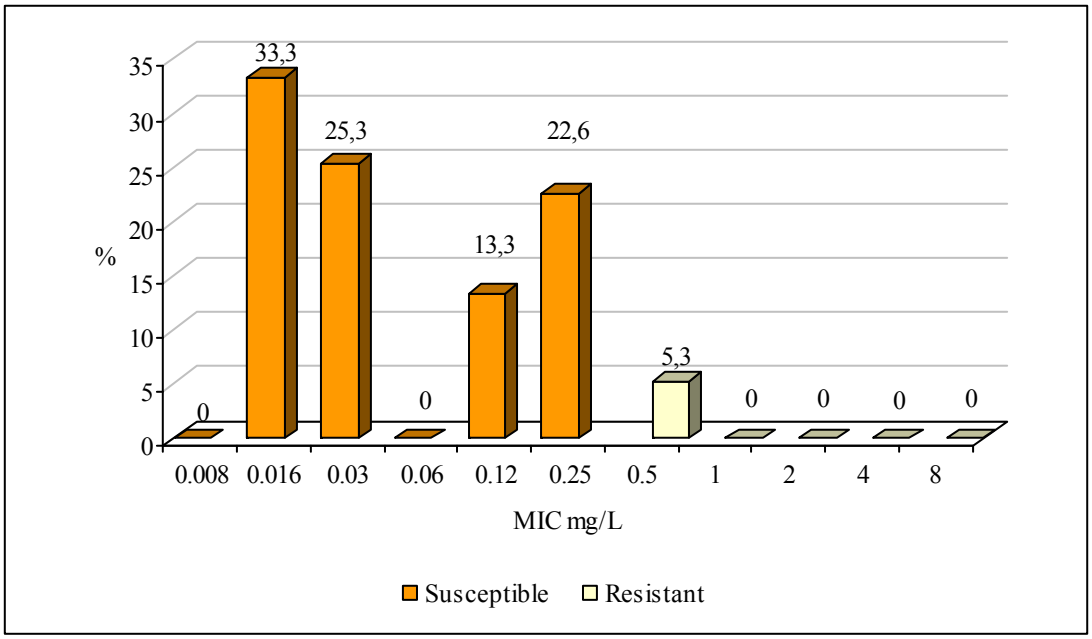

Fig. 4. **Ciprofloxacin MIC values and clinical resistance of *Salmonella enterica* isolates** (n=75)

As could be seen from Fig 3. the highest number of *Salmonella* had MIC range according to nalidixic acid at 4 mg/L. 20.0% of isolates had MIC range at 64 mg/L and the same number at 128 mg/L. Total clinical resistance of *Salmonella* to nalidixic acid was 41.3%. The results (in MIC range/percents) in "Susceptible" group indicate the 9.766 mean (M), the 16.556 (SD), the 6.759 (SEM) and the -7.610 – 27.143 (95% CI). In the "Resistant" group the results were 13.766 (M), the 10.796 (SD), the 6.233 (SEM) and the -13.055 – 40.589 (95% CI). The M of difference between "Susceptible" and "Resistant" groups was -11.100, the SD – 9.382, SEM – 5.417 and 95% CI – (-34.409 – 12.209). The linear and rank correlation between percents of susceptible and resistant groups according MIC range show that the correlation coefficient (r) was 0.389 with rank correlation (S p.r) 0.500, the p inside groups was 0.719 and 0.325, between groups – 0.177 (p>0.05, not significant).

As could be seen from Fig 4. the biggest number of *Salmonella* had MIC range for ciprofloxacin at 0.016 mg/L. Only 5.3% of *Salmonella* showed to be clinically resistant and had MIC range 0.5 g/L. In "Susceptible" group was indicate (in MIC range/percents) the 15.751 mean (M), the 13.771 (SD), the 5.622 (SEM) and the 1.295– 30.205 (95% CI) with p=0.250 (not significant). In the "Resistant" group the results were 1.061 (M), the 2.370 (SD), the 1.060 (SEM) and the -1.883 – 4.003 (95% CI) with p=0.142 (p>0.05, not significant). The M of difference between "Susceptible" and "Resistant" groups was -13.320, the SD – 16.331, SEM – 7.304 and 95% CI – (-6,955 – 33,595) with p=0.044 (statistically significant). The linear and rank correlation between percents of susceptible and resistant groups according MIC range show that the correlation coefficient (r) was 0.458 with rank correlation (S p.r) 0.388.

Epidemiological susceptibility of *Enterobacteriaceae* are shown in Table 1. As could be seen from Table 1, 53.0% of *E. coli* and 41.0% of Salmonella strains had MIC values above epidemiological cut-off values for nalidixic acid; 34.0% of *E. coli* and 41.0 % of *Salmonella* strains had MIC values above epidemiological cut-off values for ciprofloxacin.

Table 1. **Epidemiological susceptibility of *Escherichia coli* and *Salmonella enterica*,** %

Escherichia coli (n=137)				*Salmonella enterica* (n=75)			
Nalidixic acid		Ciprofloxacin		Nalidixic acid		Ciprofloxacin	
Susceptible	Resistant*	Susceptible	Resistant*	Susceptible	Resistant*	Susceptible	Resistant*
47	53	66	34	59	41	59	41

* – "resistant" means that MIC values were above natural species dependant susceptibility to certain antimicrobial (epidemiologically resistant strains).

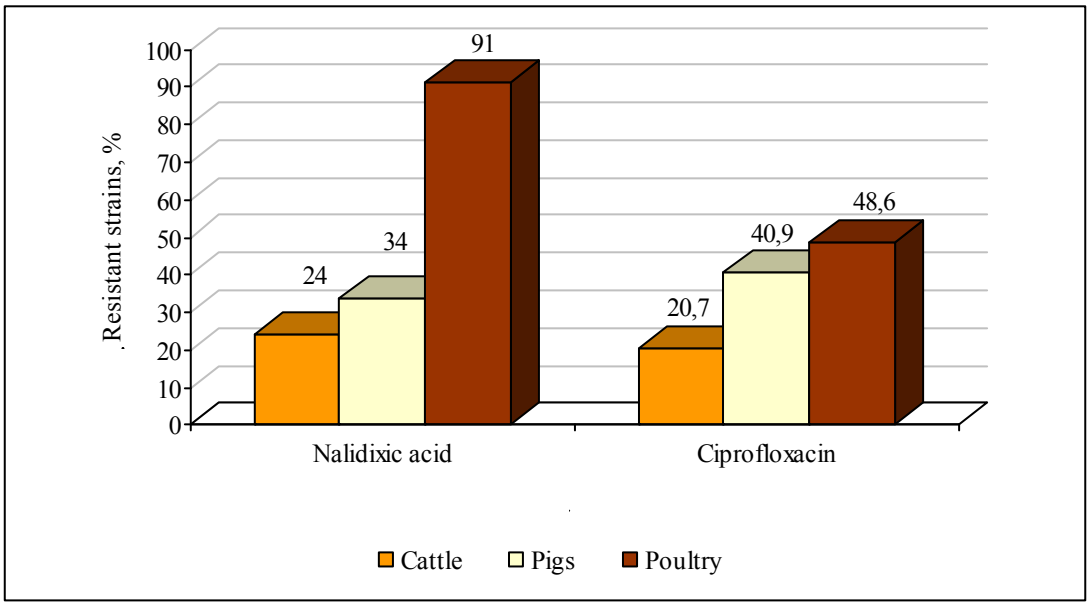

Fig. 5. **Clinical susceptibility of all tested *Escherichia coli* strains to nalidixic acid and ciprofloxacin,** % (n=137)

Clinical susceptibility of *E. coli* according to isolation origin (animal species) is demonstrated in Fig. 5. As could be seen from Fig 5. the highest resistant of *E. coli* had strains isolated from poultry:

68.6% isolates from poultry origin were resistant to nalidixic acid and 48,6 % – to ciprofloxacin. The lowest percent of resistance was among cattle isolates: 25.9% were resistant to nalidixic acid and 20.7% – to ciprofloxacin. Pig isolates had intermediate frequency of resistance. Statistically in calculation of resistant strains (%) to the nalidixic acid group were indicated next results: the 49.666 (M), the 36.143 (SD), the 20.867 (SEM) and the -40.125 – 139.46 (95% CI). In the ciprofloxacin group the results were 39.400 (M), the 16.195 (SD), the 9.350(SEM) and the -0.835 – 79.639 (95% CI). The M of difference between "nalidixic acid" and "ciprofloxacin" groups was 10.269, the SD – 29.278, SEM – 16.904 and 95% CI – (-62.471 – 83.004). The statistical investigation show that the correlation coefficient (r) was 0.355 with rank correlation (S p.r) 0.500, the p inside groups was 0.676 and 0.385, between groups – 0.167 ($p>0.05$, not significant).

Discussion. Quinolones and fluoroquinolones are effective in the treatment of serious infections like septicaemia, gastroenteritis and respiratory diseases caused by susceptible Gram-negative bacteria. They are highly potent bactericidal substances well absorbed after oral administration, have a long elimination half-life and widespread distribution throughout the body, which make them attractive to be used e.g. in herd treatment of food-producing animals (CVMP, 2007). However, the oral administration to groups or flocks of animals may have promoted the development of resistance. Knowledge about the optimal use of quinolones and fluoroquinolones could help to develop appropriate dosing regimens to reduce the selection for resistance, which would ensure their future use also for the benefit of animals (Prescott et al., 2000). In some animal pathogens resistance to other authorised antimicrobial classes like beta-lactams, tetracyclines, trimethoprim and sulphonamides is wide spread. Consequently for some diseases antimicrobial therapy will be complicated if (fluoro)quinolones loose their activity. This is a risk for animal welfare and will result in economical losses. The best documented example of this is *E. coli* septicaemia in poultry, because of the limited number of antimicrobial agents available for treatment of this animal species and the common presence of multidrug resistance (Bass et al. 1999; Blanco et al., 1997). Development of resistance may not have been expected by the early users. However, resistance to fluoroquinolones emerged and increased among several bacterial species pathogenic for food-producing animals following the introduction of enrofloxacin (Aarestrup et al., 2000). As far as *E. coli* is concerned, it has until now been the general belief that *E. coli* from animals and humans belong to different populations and that animal strains of *E. coli* usually do not infect humans, with the important exception of *E. coli* O157:H7, and other zoonotic shiga-like toxin producing *E. coli* serotypes (Aarestrup and Wegener, 1999). In Spain it has previously been reported that quinolone resistance emerged simultaneously in *E. coli* from food-producing animals and infections in humans (Garau et al., 1999). A recent study from the US has already pointed a high degree of genetic relatedness between human and animal *E. coli* (Johnson et al., 2005). The potential zoonotic origin of these *E. coli* infections is still quite uncertain and further studies are needed to clarify the matter. This study shows that 34.4% of all tested *E. coli* isolates from animal origin were resistant to ciprofloxacin – the fluoroquinolone that is commonly are used in treatment of humans. Some strains had to be more resistant than others and demonstrated very law susceptibility to both quinolones and fluoroquinolones. The same mechanism of action of different fluoroquinolones of the same generation used in human and veterinary medicine leads to resistance development in both sectors. That is the reason to pay special attention to this class of antimicrobial agents using them in animal husbandry and use them only in the cases when other classes of antimicrobial agents are ineffective. Group treatment of animals is one of the most important risk factors: the obtained results demonstrate that resistance of *E. coli* to quinolones and fluoroquinolones were more frequent in poultry isolates. Such tendency was demonstrated and in our previous studies (Ružauskas et al., 2007-1).

Similar trends have been observed for several *Salmonella* serovars. In Germany, an increase in the incidence of strains of *Salmonella* that are resistant to nalidixic acid was observed after the licensing of enrofloxacin (Malorny et al., 1999). Simultaneous increase in resistance was observed in France among isolates from animals and humans, and the same clones were observed among the different reservoirs (Heurtin-Le Corre 1999). Also in Spain the occurrence of nalidixic acid resistance among *Salmonella* causing infections in humans increased from less than 0.5% before 1991 to 38.5% in 2003 in one study (Marimon et al., 2004) and from around 6 to 15% in 1991 to 40 to 85% in 2001 in another study (Guerri Santos and Rotger, 2004). In the United Kingdom substantial increases in resistance to nalidixic acid in *Salmonella* Hadar and *Salmonella* Virchow, and in multiresistant *Salmonella* Typhimurium DT104 followed the authorisation for veterinary use of enrofloxacin in 1993 and danofloxacin in 1996 (Threlfall et al., 1997). In Taiwan a number of studies have shown the emergence of fluoroquinolone resistance in *Salmonella* from pigs and the subsequent spread of those isolates to humans (Su et al., 2001; Chiu et al., 2002; Hsueh et al., 2004). Our previous studies showed that *Salmonella enterica* spread in Lithuania in the recent years had lowest frequency of resistance to various classes of antimicrobial agents except tetracycline (Virgailis et al., 2007). This study shows that the number of resistant *Salmonella* strains to fluoroquinolones was also law. Resistance level was minimal to those antimicrobial agents (0.5 mg/L). However resistance to nalidixic acid was 41.3% ant it shows that almost half of the *Salmonella* strains has developed the first step of resistance to quinolones and have single-point gyrase gene. Our previous study on molecular genetics of *Salmonella* and *E. coli* to quinolones and fluoroquinolones showed that gene mutations of *gyrA* and

parC genes could be found in *E. coli* isolates however in *Salmonella* only mutations in gyrA gene were found (Ružauskas et al, 2006). According to those data, the situation of clinical *Salmonella* resistance to fluoroquinolones is still favourable, however all measures must be taken to keep such level of *Salmonella* resistance in future. The index of epidemiological susceptibility showed that the level of *Salmonella* susceptibility is not as law as the „normal" species level. It demonstrates possible unfavourable trends according resistance increasing. Epidemiological susceptibility of *E. coli* was comparable to the clinical resistance. However, in both cases the percentage of resistant strains of *E. coli* was high.

Analysis of antimicrobial resistance in different branches of animal husbandry indicated that resistance could be dependant on intensive and extensive usage of antimicrobial agents. Enrofloxacin is widely used in poultry farms and our results prove this theory – the biggest number of resistant isolates of *E. coli* to fluoroquinolones was determined in poultry. Statistical analysis demonstrated insignificant results according to the number of resistance strains of *E. coli* among both antimicrobial agents – nalidix acid and ciprofloxacin. Such facts are important and must be keep in mind in the case of strategy formation of usage of antimicrobial agents.

Conclusions.

1. *Escherichia coli* showed to be frequently resistant to quinolones and fluoroquinolones: 44.5% of all tested strains were clinically resistant to nalidixic acid and 34.4% – to ciprofloxacin; 22.0% of tested *E. coli* were highly resistant to nalidixic acid (MIC value – 256 mg/L) and 12.0% of the strains were highly resistant to ciprofloxacin (MIC value – 8 mg/L). Epidemiological susceptibility was similar as the clinical susceptibility according to the number of resistant strains.
2. The highest percent of resistant *E. coli* were obtained from poultry origin, the lowest – from the cattle.
3. *Salmonella enterica* demonstrated frequent clinical resistance to nalidixic acid (41,3 %) and infrequent resistance to ciprofloxacin (5.3%) with lowest MIC (0.5 mg/L). However, 41.0% of *Salmonella* had MIC values above epidemiological cut-off values both to quinolones and fluoroquinolones. This fact demonstrates possible development of resistance to fluoroquinolones.

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References

1. Aarestrup F. M., Jensen N. E., Jorsal S. E., Nielsen T. K. Emergence of resistance to fluoroquinolones among bacteria causing infections in food animals in Denmark. Veterinary Record. 2000. T. 146. P. 76–78.

2. Aarestrup F. M., Wegener H. C. The effects of antibiotic usage in food producing animals on the development of antimicrobial resistance of importance for humans in *Campylobacter* and *Escherichia coli*. Microbes and Infection. 1999. T. 1. P. 639–644.

3. Aarestrup F. M., Wiuff C., Mølbak K., Threlfall E. J. Is it time to change fluoroquinolone breakpoints for *Salmonella* spp.? Antimicrobial Agents and Chemotherapy. 2003. T. 47. P. 827–829.

4. Bass L., Liebert C.A., Lee M.D., Summers A.O., White D.G., Thayer S.G., Maurer J.J. Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. Antimicrobial Agents and Chemotherapy. 1999. T. 43. P. 2925–2929.

5. Blanco J. E., Blanco M., Mora A., Blanco J. Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. Journal of clinical microbiology, 1997. T. 35. P. 2184–2185.

6. Bywater R., Silley P., Simjee S. Antimicrobial breakpoints – definitions and conflicting requirements. Veterinary Microbiology. 2006. T. 118. P. 158–159.

7. Chiu C. H., Wu T. L., Su L. H., Chu C., Chia J. H., Kuo A. J., Chien M. S., Lin T. Y. The emergence in Taiwan of fluoroquinolone resistance in *Salmonella enterica* serotype Choleraesuis. The New England Journal of Medicine. 2002. T. 346. P. 413–419.

8. CVMP. Public statement on the use of (fluoro)quinolones in food-producing animals in the European Union: development of resistance and impact on human and animal health. European Medicines Agency. 2007.

9. Engberg J., Aarestrup F. M., Smidt P. G., Nachamkin I., Taylor D. E. Quinolone and macrolide resistance in *Campylobacter jejuni* and *coli*: A review of mechanisms and trends over time of resistance profiles in human isolates. Emerging Infectious Diseases. 2001. T. 7. P. 24–34.

10. Endtz H. P., Ruijs G. J., van Klingeren B., Jansen W. H., van der Reyden T., Mouton R.P.Quinolone resistance in campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. Journal of. Antimicrobial Chemotherapy. 1991. T. 27. P. 199–208.

11. Garau J., Xercavins M., Rodríguez-Carballeira M., Gómez-Vera J. R., Coll I., Vidal D., Llovet T., Ruíz-Bremón A. Emergence and Dissemination of Quinolone-Resistant *Escherichia coli* in the Community. Antimicrobial Agents and Chemotheapy. 1999. T**.** 43. P. 2736–2741.

12. Guerri Santos M. L., Rotger R. Change in resistance to quinolones and betalactams in different serogroups of *Salmonella* during the last decade in a Madrid hospital. Revista Española de Quimioterapia. 2004. T. 17. P. 37–43.

13. Heurtin-Le Corre C., Donnio P. Y., Perrin M.,

Travert M. F., Avril J. L. Increasing incidence and comparison of nalidixic acid-resistant *Salmonella enterica* subsp. *enterica* serotype Typhimurium isolates from humans and animals. Journal of Clinical Microbiology. 1999. T. 37. P. 266–269.

14. Hsueh P. R., Teng L. J., Tseng S. P., Chang C. F., Wan J. H., Yan J. J., Lee C. M., Chuang Y. C., Huang W. K., Yang D., Shyr J. M Yu K. W., Wang L. S., Lu J. J., Ko W. C., Wu J. J., Chang F. Y., Yang Y. C., Lau Y. J., Liu Y. C., Liu C. Y., Ho S. W., Luh K. T. Ciprofloxacin-resistant *Salmonella enterica* Typhimurium and Choleraesuis from pigs to humans, Taiwan. Emerging. Infectious. Diseases. 2004. T. 10. P. 60–68.

15. Johnson J. R., Kuskowski M. A., Smith K., O'Bryan T. T, Tatini S. Antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli* in retail foods. Journal of Infectious Diseases. 2005. T. 191. P. 1040–1049.

16. Malorny B., Schroeter A., Helmuth R. Incidence of quinolone resistance over the period 1986 to 1998 in veterinary Salmonella isolates from Germany. Antimicrobial Agents and Chemotherapy. 1999. T. 43. P. 2278–2282.

17. Marimon J. M., Gomariz M., Zigorraga C., Cilla G., Perez-Trallero E. Increasing prevalence of quinolone resistance in human nontyphoid Salmonella enterica isolates obtained in Spain from 1981 to 2003. Antimicrobial Agents and Chemotherapy. 2004. T. 48. P. 3789–3793.

18. Prescott J. F., Baggot J. D., Walker D. Antimicrobial therapy in veterinary medicine. 3 edition. Iowa State University Press. Ames, Iowa. 2000.

19. Ružauskas M., Sužiedėlienė E., Šeputienė V., Virgailis M., Šiugždinienė R., Daugelavičius R. Gyvūninės kilmės *E. coli* rūšies bakterijų fenotipinis atsparumas antimikrobinėms medžiagoms. Veterinarija ir zootechnika. 2007. T. 38. P. 61-67.

20. Ružauskas M., Virgailis M., Šiugždinienė R., Šeputienė V., Sužiedėlienė E., Špakauskas V., Daugelavičius R. Resistance of *E. coli* isolated from animal origin to quinolones and fluoroquinolones. Animal Science. Proceedings. 9 June 2007. T. 1. P. 120-121.

21. Su L. H., Chiu C. H., Kuo A. J., Chia J. H., Sun C. F., Leu H. S., Wu T. L. Secular trends in incidence and antimicrobial resistance among clinical isolates of Salmonella at a university hospital in Taiwan, 1983-1999. Epidemiology and Infection. 2001. T. 127. P. 207–213.

22. Šeputienė V., Povilonis J., Ružauskas M., Virgailis M., Žlabys P., Sužiedėlienė E. Quinolone resistance among *Salmonella enterica* and *Escherichia coli* in Lithuania. Biologija. 2006. T. 3. P. 74–78.

23. Threlfall E. J., Ward L. R., Skinner J. A., Rowe B. Increase in multiple antibiotic resistance in nontyphoidal salmonellas from humans in England and wales: a comparison of data for 1994 and 1996. Microb Drug Resist 1997. T. 3. P. 263–226.

24. Virgailis M., Ružauskas M., Šiugždinienė R., Špakauskas V., Šeputienė V., Sužiedėlienė E., Daugelavičius R. Salmonella isolation from food and it's susceptibility to tetracycline. Animal Science. Proceedings. 9 June 2007. T. 1. P. 144-145.

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