

## THE RESISTENCY OF *Salmonella* serovar. *enteritidis/infantis* ISOLATED IN POULTRY AGAINST NALIDIXIC ACID

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**Abstract:** Modern livestock production method involves the use of antimicrobial agents as an effective way to fight against various infections. The effectiveness of drugs depends on their proper and controlled use. A particular problem is bacteria, such as *Salmonella*, which are important for human and veterinary medicine. The mechanism of resistance developed in bacteria depends on the activity of antibacterial preparations. Target molecules for the group chinolone antibiotics are enzymes involved in DNA replication of cells. Quinolone bactericidal activity disrupts the function of bacterial gyrase thereby blocking DNA synthesis and causes cell death. Nalidixic acid is a prototype of quinolone, and along with several other compounds, forms the group of first and second generation of this type of antimicrobial agents. The subject of our work is to monitor the sensitivity of *Salmonella enteritidis* and *Salmonella infantis* to nalidixic acid as a method of indicating the presence of bacterial resistance to quinolones. The aim is to determine whether the resistance to mentioned nalidixic acid in *Salmonella* serovariety is present, are there differences in resistance between them and what the possible cause of these differences are. The examination was carried out on strains isolated from poultry samples. The most presented strains in both examined years were *S. enteritidis* and *S. infantis* (more than 90%). Monitoring the sensitivity of these serotypes toward nalidixic acid it was found that in 2009 *S. enteritidis* showed sensitivity with 88.46% and in 2010 year 81.25%. *S. infantis* strains showed quite a different sensitivity, i.e. the resistance to nalidixic acid. The presence of 75% resistant strains in 2009 and 68.18% resistant strains in 2010 were determined. The difference of sensitivity of isolated serovariety indicate the need to use molecular methods and try to detect not only the mechanisms of resistance but also epizootiological, epidemiological and perhaps reasons, but also the origin and circulation of strains, and determine phenotype characteristics.

**Key words:** *Salmonella enteritidis*, *Salmonella infantis*, resistance, nalidixic acid

## Introduction

Modern animal production involves continuous surveillance of animal health status in herds, but also the control of drug administration. The heavy bacterial burden in animal production is the result of antibiotic misuse. In order to increase production, optimize feed conversion and reduce losses caused by mortality, the producers use antibiotics as growth promoters, as prophylactic and therapeutic treatment (Chiu et al., 2004; Ilić et al., 2009; Stojanov et al., 2008). The effectiveness of a drug depends not only on correct and controlled use, but also if the need is real. Frequently, health problems caused by various non-bacterial diseases are treated by antibacterials for no obvious reason. Bacteria, such as *Salmonella*, present a specific problem since they play an important role both in human and veterinary medicine. *Salmonella* produces health problems in poultry, and high costs due to mortality and the applied therapy (Nogrady et al., 2007). On the other hand, *Salmonella* in poultry presents a threat to human health, so continuous control is needed. Unfortunately, the use of antibiotics, especially without expert supervision, causes the resistance of bacterial isolates. The mechanism of resistance, developed in bacteria, depends on antibacterial activity of preparations. Nalidixic acid is a base substance in the group of quinolone antimicrobial agents and, together with several other compounds, may be found in the first and second generation of antibiotics. Monitoring the presence of resistance to nalidixic acid is an indicator of reduced sensitivity of bacteria isolated in fluoroquinolone (Iciar et al., 2005). Since 1962, when synthesized for the first time, up to nowadays, more than 10,000 substances belonging to this group of antibiotics have been produced. Target molecules for the group of quinolone antibiotics are enzymes involved in DNA cell replication. The bactericidal effect of quinolone inhibits the function of bacterial gyrase, thereby blocks DNA synthesis and causes cell death. The objective of our work was to monitor the sensitivity to nalidixic acid in two most isolated serotypes: *Salmonella enteritidis* and *Salmonella infantis*. The data obtained in this way point on bacteria resistant to quinolone antibiotic group. The aim of this work was to determine whether the resistance to nalidixic acid is present in the above mentioned *Salmonella* serovarieties, if they express differences in resistance and what the possible cause of these differences is.

## Materials and Methods

The investigation was carried for two years. In this period all the samples arriving at the laboratory were tested for the presence of *Salmonella*. Isolated *Salmonella* strains were serotyped and their sensitivity was examined to the antibiotic group, including nalidixic acid.

The samples were tested by standard laboratory methods using peptone broth as a base for pre-enrichment and modified semisolid Rappaport vasiladis medium (MSRV), (HiMedia) incubated at 42°C. After 24 hours the samples were incubated and transferred by loop from a medium with MSR to a *Salmonella* differential agar (HiMedia). All transparent colonies with a red peak in the middle were streaked into a triple sugar (Kligler agar (HiMedia)) (Quinn *et al.* 2002). The isolates were tested by biochemical sequence and poly, group and phase sera for *Salmonella*. Antibiotic sensitivity testing was performed by disk diffusion method, according to CLSI protocol.

## Results and Discussion

In 2009 we examined 1745 samples from poultry, out of which 71 were positive on the presence of *Salmonella*. In 2010 a total of 1832 samples was examined, out of which 114 were positive on *Salmonella*. In Table 1 are displayed data on the number of isolated and detected *Salmonella* serotypes. All isolated serovar *Salmonella* belong to *Salmonella Enterica*.

**Table 1. *Salmonella* serotypes isolated in 2009 and 2010**

	2009	2010
Total number of samples	1745	1832
<i>Salmonella</i> serovar. <i>enteritidis</i>	44 (2.52%)	64 (3.49%)
<i>Salmonella</i> serovar. <i>infantis</i>	20 (1.46%)	44 (2.40%)
<i>Salmonella</i> serovar. Typhimurium	5 (0.29%)	3 (0.16%)
<i>Salmonella</i> serovar. <i>hadar</i>	2 (0.11%)	3 (0.16%)
Total number of isolated <i>Salmonella</i>	71 (4.07%)	114 (6.22%)

In Table 1 it can be seen that the most common serovars in this period were *S. enteritidis* and *S. infantis*. In 2009 it accounted for 90.14% of all *Salmonella* isolates, but 2010 it was 91.22%. When compared with the data from

literature (Carraminana et al., 2004) it can be seen that in other countries *S. enteritidis* is most frequently isolated *Salmonella*. In some countries *S. infantis* cannot be found in the material originating from poultry (Muhammada et al., 2010) or it is present in very small quantity (1.9%) (Frye et al. 2007). In contrast to these data in the work (Ohad et al., 2010) it can be seen that the presence of *S. infantis* has increased since 2006 (both in humans and poultry), but since 2008 *S. infantis* has taken precedence and become the most common serotype. It became more ubiquitous than previously *S. enteritidis*.

In Table 2 are given the data on sensitivity to nalidixic acid two most represented serovars: *S. enteritidis* and *S. infantis*.

**Table 2. Sensitivity of *S. enteritidis* and *S. infantis* to nalidixic acid**

	2009		2010	
	<i>S. enteritidis</i>	<i>S. infantis</i>	<i>S. enteritidis</i>	<i>S. infantis</i>
Resistant	4 (9.09%)	15 (75%)	9 (14.06%)	30 (68.18%)
Intermediate	1 (2.27%)	1 (5%)	3 (4.69%)	-
Sensitive	39 (88.64%)	4 (20%)	52 (81.25%)	14 (31.81%)
Total	44	20	64	44

The authors of the paper (Iciar et al., 2005), who analysed sensitivity of bacterial isolates of human origin to fluoroquinolone, detected the increase of nontyphoidal *Salmonella* strains from 1.6% in 1993 to 34% in 1998. Our research in two years showed that the decrease in the sensitivity of *S. enteritidis* to nalidixic acid is obvious and that the resistance was 5% higher in 2010 than in 2009. The testing carried out in Hungary in 2007 (Nogrady et al., 2007) showed that *S. infantis* is a serotype that is frequently found in the samples originating from poultry. In humans it is the third most common cause of food poisoning. In addition, there was a significant increase in resistance of *S. infantis* to nalidixic acid in isolates originating from humans and poultry (Nogrady et al., 2008). It was found that 72% of isolated strains were resistant to more antibiotics (multidrug-resistant MDR) (nalidixic acid-streptomycin-sulphonamide-tetracycline). In 2005 the resistance to nalidixic acid was complete. The results of the paper (Avsaroglu et al., 2007), in which are analyzed isolates originating from poultry and poultry meat, indicate that all *S. infantis* serotypes were resistant to nalidixic acid and significantly reduced their susceptibility to ciprofloxacin.

Modern production and the possibility of rapid transport of live animals or poultry meat products does not seem to contribute equally to the spread of individual *Salomonela* serotypes and the spread of their resistance. The paper by Musgrove (Musgrove et al., 2006) reports that present multi-resistance of isolated

strains of *Salmonella* depends on the serotype. The most frequently isolated serotypes were *S. typhimurium* and *S. kentucky*. The resistance to nalidixic acid was 63.4%. Similar to this, the findings in Turkey (Erdema et al., 2005) report that in human samples a very small percentage of *Salmonella* was isolated from C group (8.5%) out of which only 3.77% were *S. infantis*. The resistance to cyprofloxacin was not observed. The researchers from Nigeria report that in the samples from poultry *S. infantis* and *S. enteritidis* could not be found, but the most common serotype was *S. virchow*. The authors note that multidrug-resistance was high (62%) and that in addition to resistance to nalidixic acid a decreased sensitivity of *S. virchow* was noted to trimethoprim, sulfamethoxazole and tetracycline (Kayode et al., 2010). In Japan (Kanako et al., 2009) *S. infantis* was the most prevalent serotype (22.6%) in poultry, but resistance to nalidixic acid was only 5%. All these data point to significant differences in the presence of certain *Salmonella* serotypes and different distribution of resistance throughout the world.

## Conclusion

The collected data indicate that in two year period *S. enteritidis* and *S. infantis* strains accounted for the largest portion of total isolated *Salmonella* serotypes. In 2009 these two serotypes accounted for 90.14% of all *Salmonella* isolates, and in 2010 they accounted for 91.22%. Investigating the sensitivity of these strains to nalidixic acid, it was detected that in 2009 less than 10% of *S. enteritidis* strains showed resistance to this antibiotic, while in 2010 it was 14.06%. As for as for the *S. infantis* it can be seen that in both years the percentage of resistant strains was very high and ranged around 70%. There is no answer why such a high percentage of resistance of *S. infantis* to nalidixic acid. This resistance, however, can create problems in the application of antibiotics in the treatment group fluoroquinolone in humans in the case of illness caused by these serotypes as well as the treatment of poultry. Applying the resistance tests at molecular level and cooperation with neighboring countries, may contribute to a better understanding of the problem and provide a way for finding a resolution.

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## Rezistencija *Salmonella* serovar. *enteritidis/infantis* izolovanih kod živine prema nalidiksičnoj kiselini

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### Rezime

Savremeni način stočarske proizvodnje podrazumeva upotrebu antimikrobnih lekova kao efikasan način borbe protiv različitih infekcija. Efikasnost lekova zavisi od njihovog pravilnog i kontrolisanog korišćenja. Poseban problem predstavljaju bakterije, kao što je salmonela, koje su značajne i za humunu i za veterinarsku medicinu. Mehanizam rezistencije koji se razvija kod bakterija zavisi od antibakterijskog delovanja preparata. Ciljni molekuli za grupu hinolonskih antibiotika su enzimi koji učestvuju u replikaciju DNK ćelije. Baktericidno delovanje hinolona remeti funkcije bakterijske giraze čime se blokira sinteza DNK i izaziva smrt ćelije. Nalidiksična kiselina predstavlja prototip svih hinolona i zajedno sa nekoliko drugih jedinjenja nalazi se u grupi prve i druge generacije ove vrste antimikrobnih lekova. Predmet našeg rada je praćenje osetljivosti *Salmonella enteritidis* i *Salmonella infantis* prema nalidiksičnoj kiselini kao metodi koja ukazuju na prisustvo rezistencije bakterija na hinolone. Cilj rada je da utvrdimo da li je prisutna rezistencija na nalidiksičnu kiselinu kod spomenutih serovarijeteta salmonela, da li postoje razlike u rezistenciji među njima i šta je mogući uzrok ovih razlika. Isptivanje je obavljeno na sojevima izolovanim iz živinskih materijala. Najzastupljeniji sojevi u obe posmatrane godine su bili *S. enteritidis* i *S. infantis* (nešto više od 90%). Praćenjem osetljivosti ova dva serotipa prema nalidiksičnoj kiselini utvrđena je u 2009. godini osetljivost *S. enteritidis* 88,46% a u 2010. godini 81,25%, dok je rezistencija *S. infantis* bila velika 75%, odnosno 68,18% u posmatranom periodu. Utvrđene razlike osetljivosti izolovanih serovarijeteta ukazuju na potrebu da se molekularnim metodama pokušaju sagledati ne samo mehanizmi nastanka rezistencije već i epizootiološko, a možda i epidemiološko poreklo i kruženje sojeva, odnosno njihovih rezistentnih fenotipskih osobina.

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