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SEROTYPING AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA* SPP. ISOLATED FROM COMMERCIAL FLOCKS OF LAYING HENS IN R. MACEDONIA

Prodanov Mirko[1], Ratkova Marija[1], Angelovski Ljupco[1], Jankuloski Dean[1], Sekulovski Pavle[1], Mrenoshki Slavcho[2]

[1]*Food Institute, Faculty of Veterinary Medicine, University of ss. Cyril and Methodius in Skopje*
[2]*Veterinary Institute, Faculty of Veterinary Medicine, University of ss. Cyril and Methodius in Skopje*

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ABSTRACT

In the period from October to December 2011, 61 isolates of *Salmonella* were isolated from flocks of laying hens providing eggs for human consumption. Most of the isolates belonged to the serovars Enteritidis (n=35; 57.38%), Typhimurium (n=17; 27.87%) and other (n=9; 14.75%). These isolates were tested for antimicrobial resistance to ampicillin, cefotaxime, chloramphenicol, ciprofloxacin, nalidixic acid, sulfamethoxazole, tetracycline, gentamicin and streptomycin. All of the tested *Salmonella* were sensitive to cefotaxime, chloramphenicol and ciprofloxacin. The highest resistance rate was shown to tetracycline with eight (13,11%) and streptomycin with nine (14,75%) isolates, three (4,92%) isolates were resistant to nalidixic acid, and one isolate (1,63%) exhibited resistance to ampicillin, sulfamethoxazole and gentamicin. Forty four isolates (72,13%) were sensitive to all antimicrobials. i.e. 17 (27,87%) isolates showed resistance to at least one antimicrobial of which 12 isolates showed resistance to one, four isolates showed resistance to two antimicrobials and one isolate showed resistance on three antimicrobials. When results of resistance levels of *Salmonella* spp. were compared with the official data from the European Union reported by EFSA, it was concluded that the *Salmonella* spp. isolated from flocks of laying hens providing eggs for human consumption in R. Macedonia have low resistance to antimicrobial drugs.

Keywords: *Salmonella*, antimicrobial resistance, poultry

INTRODUCTION

The number of cases of salmonellosis in humans has shown significant decline over the past five years, according to the report by the European Food Safety Agency (1) and the European Centre for Disease Prevention and Control (2), yet it remains among the most common zoonotic diseases.

Implementation of the European Commission (EC) regulations to reduce specific *Salmonella* serovars in flocks of laying hens of the species *Gallus gallus* (3) through the National Control Programme, led to a significant drop in the prevalence of infection with *S.* Enteritidis and *S.* Typhimurium. Subsequently, a reduction in the incidence of human infections with *S.* Enteritidis, which is most commonly found in eggs, was reported (1). However, in 35,4% of all reported and confirmed cases of food poisoning, the source was *Salmonella* spp. and the food poisoning was usually attributed to table eggs (23,1%), pork and meat products (10,2%) and meals in restaurants (9,2%) (1). In the EU, food poisoning caused by eggs, egg products and pastry were linked to *S.* Enteritidis, while *S.* Typhimurium was most commonly found in pork meat.

The EC regulation No 1177/2006 concerning the prohibition of the use of antibiotics as a specific method of *Salmonella* control in poultry (4) was also implemented in the National Programme in Macedonia. The regulation allows use of antibiotics

Corresponding author: Mirko Prodanov, MSc

E-mail address: m.prodanov@fvm.ukim.edu.mk
Present address: Food institute, Faculty of Veterinary medicine-Skopje, "Ss. Cyril and Methodius" University,
Lazar Pop- Trajkov 5-7, 1000 Skopje, R. Macedonia
tel: +389 2 3240 734; fax: +389 2 3114 619

only in individual cases. In addition to mandatory data collection on *Salmonella*, monitoring of the antimicrobial resistance is in practice. *Salmonella* still causes food poisoning worldwide and in sensitive animal populations is also frequently prescribed (5). In a previous study on antimicrobial resistance of *Salmonella* spp. in R. Macedonia the importance of continuous monitoring of food pathogens in the whole food chain was emphasized (6).

The goal of this study was to determine the serotype of 61 isolates of *Salmonella* spp. from flocks of laying hens of the species *Gallus gallus* in R. Macedonia, and to determine antimicrobial resistance to several classes of antibiotics.

MATERIAL AND METHODS

Salmonella was isolated from fecal and dust samples from 111 flocks of laying hens providing eggs for human consumption during the laying period. From each flock two of fecal and one of dust sample was collected. The isolation method used was ISO 6579: 2002/Amd1:2007 (7). Sixty one isolate were included in the study.

The serotyping was done by slide agglutination (8) by polyvalent and monovalent antisera (Biorad, Hercules, California, U.S.).

Antibiotic resistance was determined with the disc diffusion method according to Kirby-Bauer. The isolates were tested with the following antimicrobial discs: ampicillin 10μg, cefotaxime 30μg, chloramphenicol 30μg, ciprofloxacin 5μg, nalidixic acid 30μg, sulfamethoxazole 25μg, tetracycline 30μg, gentamicin 10μg and streptomycin 10μg, (Oxoid, Hampshire, England). The discs were placed on a surface of a Müller-Hinton agar inoculated with suspension of the tested isolate. The plates were incubated at 37°C for 20 hours. After the incubation the diameter of the inhibition zone was measured in mm and the results were interpreted as resistant, intermediate or sensitive according to the Clinical Laboratory Standards Institute manual M100-S22 from 2012 (9).

RESULTS

During the period from October to December of 2011, the antimicrobial resistance of 61 strains of *Salmonella*, which were isolated from samples of laying hens, was tested. From the isolated strains 35 were *S*. Enteritidis, 17 were *S*. Typhimurium, 3 were *S*. Infantis, 2 were *S*. Derby and there was 1 strain of *S*. Heidelberg, *S*. Livingstone and *S*. Typhi. One isolate of *Salmonella* couldn't be serotyped.

All 35 isolates of *S*. Enteritidis were sensitive to cefotaxime, chloramphenicol, ciprofloxacin and gentamicin. A total of 3 strains were resistant to tetracycline, 2 strains to streptomycin and 1 strain to nalidixic acid (Table 2).

Of the 17 strains of *S*. Typhimurium all the isolates were sensitive to ampicillin, cefotaxime, chloramphenicol, ciprofloxacin, nalidixic acid and sulfamethoxazole. A total of 7 isolates were resistant to streptomycin, 2 isolates were resistant to tetracycline and 1 isolate was resistant to gentamicin (Table 3).

All the isolates other than *S*. Enteritidis or *S*. Typhimurium were sensitive to cefotaxime, chloramphenicol, ciprofloxacin, sulfamethoxazole, gentamicin and streptomycin. A total of 3 isolates were resistant to tetracycline, 2 were resistant to nalidixic acid, and 1 was resistant to ampicillin (Table 4).

None of isolates in this study exhibited resistance to cefotaxime, chloramphenicol and ciprofloxacin. Nine isolates were resistant to streptomycin, 8 were resistant to tetracycline, 3 were resistant to nalidixic acid, 1 strain was resistant to ampicillin, 1 to sulfamethoxazole and one was resistant to gentamicin (Table 1).

Forty-four (72.13%) of all isolates were sensitive to all of the antimicrobials included in this study. In terms of number of antimicrobials to which individual isolates were resistant, the remaining 17 strains (27.87%) exhibited resistance to at least one antimicrobial of which 12 isolates exhibited resistance to one, 4 isolates exhibited resistance to two antimicrobials and 1 isolate exhibited resistance to three antimicrobials (Table 5).

Table 1. Antimicrobial resistance for all the isolates of *Salmonella* spp. (n=61)

Antimicrobial	No. of resistant isolates	% resistant isolates
Ampicillin	1	1.64%
Cefotaxime	0	0.00%
Chloramphenicol	0	0.00%
Ciprofloxacin	0	0.00%
Nalidixic acid	3	4.92%
Sulfamethoxazole	1	1.64%
Tetracycline	8	13.11%
Gentamicin	1	1.64%
Streptomycin	9	14.75%

Table 2. Antimicrobial resistance of *Salmonella* Enteritidis. (n=35)

Antimicrobial	No. of resistant isolates	% resistant isolates
Ampicillin	0	0.00%
Cefotaxime	0	0.00%
Chloramphenicol	0	0.00%
Ciprofloxacin	0	0.00%
Nalidixic acid	1	2.86%
Sulfamethoxazole	1	2.86%
Tetracycline	3	8.57%
Gentamicin	0	0.00%
Streptomycin	2	5.71%

Table 3. Antimicrobial resistance of *Salmonella* Typhimurium. (n=17)

Antimicrobial	No. of resistant isolates	% resistant isolates
Ampicillin	0	0.00%
Cefotaxime	0	0.00%
Chloramphenicol	0	0.00%
Ciprofloxacin	0	0.00%
Nalidixic acid	0	0.00%
Sulfamethoxazole	0	0.00%
Tetracycline	2	11.76%
Gentamicin	1	5.88%
Streptomycin	7	41.18%

Table 4. Antimicrobial resistance for all the isolates that were not *S.* Enteritidis or *S.* Typhimurium (n=9)

Antimicrobial	No. of resistant isolates	% resistant isolates
Ampicillin	1	11.11%
Cefotaxime	0	0.00%
Chloramphenicol	0	0.00%
Ciprofloxacin	0	0.00%
Nalidixic acid	2	22.22%
Sulfamethoxazole	0	0.00%
Tetracycline	3	33.33%
Gentamicin	0	0.00%
Streptomycin	0	0.00%

Table 5. Resistance pattern of *Salmonella* isolates resistant to more than one antibiotic

Serotype	Resistance pattern
S. Enteritidis	ampicillin / nalidixic acid/ tetracycline
S. Enteritidis	streptomycin / tetracycline
S. Enteritidis	gentamicin/ streptomycin
S. Infantis	nalidixic acid/ tetracycline
S. Derby	streptomycin/ tetracycline

DISCUSSION

In this study we have shown that a small percentage of isolates exhibited antimicrobial resistance. According to the EU regulation 37/2010, eggs placed on the market must be free of residues of antimicrobials, which limit their use in layers to the period before the onset of lay (10). The obtained results show low level of antimicrobial resistance, similar to other studies that are dealing with isolates of *Salmonella* spp. from laying hens (11-13). Indeed, *Salmonella* isolated from laying hens is less resistant to antimicrobial agents comparing to *Salmonella* from broilers, pigs and cows (14-16).

In this study the highest resistance was to tetracycline and streptomycin with 8 and 9 resistant isolates, respectively. According to the literature the antimicrobial resistance to those two antimicrobials is common for *Salmonella* spp. isolated from poultry products (17, 18). In comparison, in the EU the overall resistance to tetracycline was 14% and varied between 0% and 25% across the reporting member states.

The resistance to nalidixic acid in this study was found in 3 isolates all of which belonged to different serotypes. This resistance rate was much lower than the EU overall resistance rate for nalidixic acid of 18%. The resistance rate to nalidixic acid in EU countries varied between 0% and 38% (19).

Resistance to gentamicin, ampicillin and sulfamethoxazole was observed only once and within different isolates of *Salmonella* spp. In the EU reported resistance to gentamicin was very low (0,2% to 5%) in the member states; ampicillin and sulfamethoxazole resistance rate was 12% and 16%, respectively, and ranged from 0% to 36% and 0% to 36%, respectively (19).

All the *Salmonella* spp. isolates were susceptible to chloramphenicol, cefotaxime and ciprofloxacin. In the EU antimicrobial resistance to chloramphenicol, cefotaxime and ciprofloxacin was 3%, 2% and 18%, respectively (19).

The results of this study show low rate of antimicrobial resistance of *Salmonella* spp. isolates from laying hens in R. Macedonia. Nevertheless it is necessary to abide to the principles of rational use of antimicrobials in poultry breeding, to avoid increased bacterial resistance.

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