



Antimicrobial susceptibility of *Salmonella* spp. strains isolated from Layer Hens in Campania Region from 2000 to 2003

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ABSTRACT

The aim of this study was to determine the antimicrobial resistance in 60 *Salmonella* strains (*S. enteritidis*, *S. typhimurium*, *S. gallinarum*) isolated from layer hens in Campania region from 2000 to 2003. *S. gallinarum* showed resistance against ciprofloxacin and enrofloxacin, in contrast, *S. enteritidis* and *S. typhimurium* were fully susceptible. In all of isolates high levels of resistance were observed for neomycin, gentamicin and oxytetracycline. Also, one significant observation was that all of the isolates showed full susceptibility to Sulphamethoxazole/Trimethoprim. These results suggest importance to restrict the use of antibiotics in layers hens flocks in order to reduce the selection and spread of multiresistant strains.

Key Words: Layer Hens, Antimicrobial resistance, *Salmonella*, Campania region.

RIASSUNTO

SENSIBILITÀ AGLI ANTIBIOTICI DI CEPPI DI SALMONELLA SPP. ISOLATI DA GALLINE OVAIOLE IN CAMPANIA NEL TRIENNIO 2000/2003

Scopo del presente lavoro è stato quello di testare la sensibilità antibiotica di 60 ceppi di Salmonella (S. enteritidis, S. typhimurium, S. gallinarum) isolati da galline ovaiole nel periodo compreso tra il 2000 e il 2003. S. gallinarum mostrava resistenza nei confronti di ciprofloxacina ed enrofloxacina (rispettivamente 15% e 23%), al contrario di S. enteritidis e S. typhimurium che manifestavano una completa sensibilità. Tutti i sierotipi valutati presentavano alte percentuali di resistenza nei confronti di neomicina, gentamicina e ossitetraciclina. Nei confronti dei sulfamidici i ceppi testati presentavano resistenza nulla. Tali risultati suggeriscono un uso più moderato e mirato degli antibiotici negli allevamenti in modo da ridurre la selezione e diffusione di ceppi multiresistenti.

Parole chiave: Galline ovaiole, Antibiotico-resistenza, *Salmonella*, Campania.

Introduction

Antimicrobial resistance is the capacity of bacteria to survive exposure to a defined concentration of an antimicrobial substance. It is the natural response of bacterium to defend itself against the effects of an antibiotic. The development of antimicrobial resistance is an ecological phenomenon. Any antibiotic use, whether in

humans, animals or plants/environment may lead to resistance (OIE, 2003).

The extensive use of antibiotics, not only in human and veterinary medicine, but also in livestock production for disease prevention or as growth-promoting feed additives, has led to a serious increase in, and spread of, multiple antibiotic-resistant bacteria (Cruchaga *et al.*, 2001).

All this caused considerable problems to

approach prophylactics and therapeutics plans versus various bacterial pathologies.

Salmonellosis has a particular role in avian medicine whether host-specific serotypes (*S. gallinarum*, *S. pullorum*) or non-host specific serotypes (*S. enteritidis*, *S. typhimurium*) implicated in food-borne zoonoses.

Within the routine control programmes carried out in the poultry farms organized from the Avian Pathology Section of the Dipartimento di Patologia e Sanità Animale in University of Bologna, Italy; the *Salmonella* strains isolated showed an increase of antibiotic resistance pattern.

The aim of this study was to determine the antimicrobial resistance in *Salmonella* strains (*S. enteritidis*, *S. typhimurium*, *S. gallinarum*) isolated from layer hens in Campania region from 2000 to 2003.

Material and methods

Sample collection

From January 2000 to November 2003 a total of 60 *Salmonella* strains were isolated from layer hens flocks, respectively belonged to *S. gallinarum*, *S. enteritidis*, *S. typhimurium*. The strains collected were 20 for each serotype.

Isolation and identification procedure

The *Salmonella* isolation procedures were carried out following the WHO standard methods (WHO, 1994). All the strains were serotyped at National Reference Centre for *Salmonella* (Istituto Zooprofilattico Sperimentale delle Venezie, Padova - Italy).

Antimicrobial susceptibility tests

Antimicrobial susceptibility profiles of the isolates were determined by the disk diffusion method according to the NCCLS guidelines (National Committee for Clinical Laboratory Standards, 2002). The antimicrobial agents (Oxoid) tested and corresponding concentration were as follows: Ciprofloxacin 5µg (C), Enrofloxacin 5µg (E), Flumequine 30µg (F), Nalidixic acid 30µg (NA), Apramycin 15µg (AP), Amoxicillin 10µg (A), Neomycin 30µg (N), Gentamicin 10µg (G), Oxytetracycline 30µg (O), Sulphamethoxazole/Trimethoprim 25µg (S/T).

The diameters of the inhibition zone for the interpretation of resistance and susceptibility were those recommended by the NCCLS (National Committee for Clinical Laboratory, 2002). Results were scored as susceptible, moderately susceptible or resistant according to NCCLS criteria (2002). The reference strain used was *Escherichia coli* ATCC 25 922.

Results and discussion

As seen from Table 1, resistance of *S. gallinarum* was significantly higher than other two serotypes examined. In particular, *S. gallinarum* showed resistance against two fluoroquinolone (ciprofloxacin and enrofloxacin, respectively 15% and 23%), in contrast, *S. enteritidis* and *S. typhimurium* were fully susceptible. In all of isolates high levels of resistance were observed for neomycin, gentamicin and oxytetracycline. It was also found that *S. enteritidis* and *S. gallinarum* were resistant to apramycin (33.3% and 38.5% respectively) and *S. gallinarum* was resistant

Table 1. Antimicrobial resistance of 60 *Salmonella* spp. isolates.

Serotype	n.	Antimicrobial resistance (%) against antimicrobial agents tested									
		C	E	NA	AP	A	N	G	F	S/T	O
<i>S. enteritidis</i>	20	0	0	6	33.3	0	55.5	38.8	5.5	0	33.3
<i>S. typhimurium</i>	20	0	0	13	0	0	12.5	25	12.5	0	50
<i>S. gallinarum</i>	20	15	23	15	38.5	23.1	53.8	55.3	23.1	0	46.1

C=Ciprofloxacin, E=Enrofloxacin, F=Flumequine, NA=Nalidixic acid, AP=Apramycin, A=Amoxicillin, N=Neomycin, G=Gentamicin, O=Oxytetracycline, S/T=Sulphamethoxazole/Trimethoprim

to amoxicillin too (23.1%). In conclusion, one significant observation was that all of isolates showed full susceptibility to Sulphamethoxazole/Trimethoprim.

An increase in the incidence of antibiotic resistance in *Salmonella* isolated from humans and animals related to exhaustive application of antibiotics in both groups has been documented worldwide (Chruchaga *et al.*, 2001). Recently, Lee *et al.* (Lee *et al.*, 2003) reported, in an antimicrobial susceptibility test against 258 isolates of *S. gallinarum*, a reduced susceptibility to ampicillin (13.0%), gentamicin (43.4%), kanamycin (69.6%), enrofloxacin (6.5%), ciprofloxacin (10.9%), norfloxacin (52.5%) and ofloxacin (82.6%). A study on antimicrobial-resistant *Salmonella enterica* serovars isolated from chickens in Spain showed high percentage of resistance to chloramphenicol (44.6%), ampicillin (34.8%) and tetracycline (33.9%) (Hernandez *et al.*, 2002). Jones *et al.* (2002) reported *S. typhimurium* strains resistant to ampicillin, sulphonamides, streptomycin, chloramphenicol and tetracyclines as well as *S. typhimurium* isolated from poultry resistant to nalidixic acid (Jones *et al.*, 2002).

Fluoroquinolones resistance was rarely found among *Salmonella* species until Heisig reported *S. typhimurium* serovar *Copenhagen* from cattle was highly resistant to ciprofloxacin (Heisig, 1993).

Conclusions

An important finding is the antimicrobial resistance observed in *S. gallinarum* against fluoroquinolones (ciprofloxacin and enrofloxacin). High percentage of resistance observed in *S. typhimurium* and *S. enteritidis* for neomycin, gentamicin and oxytetracycline demonstrate improper use of these antibiotics in the control of avian salmonellosis, particularly in metaphylactic sense.

These results confirm importance to restrict the use of antibiotics in layers hens flocks in order to reduce the selection and spread of multiresistant strains and underlines the need for integrated surveillance systems of antibiotic resistance that consider isolates not only from human disease but also from the animal reservoirs and the food vehicles.

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