Salmonella Serovars and Antimicrobial Resistance Profiles in Commercial Layer Flocks

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ABSTRACT

Salmonellosis is a worldwide problem for both poultry and human health. Uninformed, common usage of antibiotics in commercial layer flocks and circulation of antibiotic resistance patterns from flock to flock is an obstacle to combat Salmonellosis. Transmission of resistant paratyphoid *Salmonella*e to people by consumption of chicken meat, egg and chicken by-products which are prepared under inappropriate conditions or undercooked, induces a transfer of antibiotic resistance patterns. The purpose of the study was to determine the *Salmonella* prevalence, distribution of *Salmonella* serovars in different ages of laying hens, and also antibiotic resistance profiles of the serovars from birds which appear healthy in the Bandirma Region of Turkey, where commercial laying hen breeding was intensively performed. Isolation was performed according to ISO 6579:2002/Amd 1:2007 (Annex D) and isolated *Salmonella* strains were serotyped according to Kauffmann-White scheme. Of the examined randomly collected 362 fecal samples, 45 (12.4%) were found to be *Salmonella* positive. *S. Infantis*, *S. Kentucky, S. Enteritidis, S. Mbandaka* were found to be common serovars. A total 45 *Salmonella* isolates was tested for susceptibility to 10 antibiotics by Kirby Bauer Disk Diffusion Method. Approximately fifty-three percent (24/45 of *Salmonella* isolates) were found to be resistant to one or more of the antibiotics. Of the tested antibiotics, none of the isolates exhibited resistance to ciprofloxacin and gentamycin while the highest resistance was found in tetracycline, the lowest resistance was determined in ciprofloxacin. All *S. Mbandaka* isolates were determined to be resistant solely to kanamycin. The other serovars were determined to be multi-drug resistanct (MDR). In summary, when the results of the study were evaluated it was judged that there was a need for effective vaccination programs, precautions of biosecurity planned for each flock and also application of concious antibiotic usage inorder to protect both poultry and human health.

Keywords: Salmonella serovars; Commercial layer flock; Feces; Antibiotic resistance.

INTRODUCTION

Salmonella is a significant problem for public health and the poultry industry. Many of *Salmonella* outbreaks in humans are related to consumption of contaminated foods containing poultry products, such as poultry meat and eggs. Therefore, specific preventive strategies for *Salmonella* should be undertaken in the poultry industry with consideration given to protect public health. Commercial layer farms can

be a significant reservoir of *Salmonella* infection and pose a threat to humans (1, 2, 3). Harker *et al.* (4) reported human salmonellosis outbreaks originating through the consumption of contaminated eggs in England and Wales between 2000 and 2011. The contamination of eggs with *Salmonella* can occurr through the laying hens by vertical or horizontal transmission (5, 6). Many studies worldwide and in Turkey have been conducted on layer farms to determine the prevaleance of *Salmonella* (7, 8, 9, 10, 11). Therefore laying hen farms should be managed to diminish prevalence of *Salmonella* in the chickens and eggs by either antibiotic treatment, effective vaccination and sanitation. Due to uninformed antibiotic usage in commercial layer chickens, appearance of multi-drug resistant microorganisms and transmission of resistance patterns to human via ingesting eggs, Salmonellosis is a growing health concern (12).

In this study it was designed to determine the dominat *Salmonella* serovars circulating in commercial layer chickens in Bandirma and to ascertain the resistance profiles of serovars against commonly used antibiotics in the field.

MATERIAL AND METHODS

Samples

Between August 2013 and February 2014, a total of 362 fresh fecal samples were randomly collected from 9 commercial layer flocks in Bandirma situated in Northwestern Turkey. The summarized information about nine commercial layer flocks is presented (Table 1). The samples were transported to the laboratory under cold chain on the day of sampling.

Bacteriology

The feacal samples were examined according to ISO 6579:2002/Amd 1:2007 (annex D) on the day of transport (13).

Serotyping

Salmonella isolates were serotyped according to the Kauffmann-White Scheme (14).

Antibiogram

Agar Disk Diffusion Method was performed according to Clinical and Laboratory Standards Institute (CLSI) (15). The following antibiotic disks were used: Ampicillin (AMP, 10µg, Oxoid CT0003B), Chloramphenicol (C, 30µg, Oxoid CT0013B), Ciprofloxacin (CIP, 5µg,Oxoid CT0425B), Gentamicin (CN, 10µg, Oxoid CT0024B), Kanamycin (K, 5µg, Oxoid CT0025B)(K), Nalidixic acid (NA, 30µg, Oxoid CT0031B), Streptomycin (S, 10µg, Oxoid CT0047B),Trimethoprim/sulfamethoxazole (SXT, 1.25/23.75µg, Oxoid CT0052B), Tetracycline (TE, 10µg, Oxoid CT0053B), Trimethoprim (W, 5µg, Oxoid CT0076B).

E. coli (ATCC 25922) and *S. Aureus* (ATCC 25923) standard strains were used as controls according to CLSI (15). Strains were evaluated as susceptible, intermediate or resistant. Multiple drug resistance (MDR) was defined as resistance to two or more agents.

Table 1: Information about the flocks and collected samples

Flock No	Race	Age (week)	Number of total chickens in flock	Number of collected samples
1	Lohmann	35	17.430	38
2	Lohmann	37	20.680	42
3	Hy-Line	38	19.712	41
4	Lohmann	43	22.141	41
5	Hy-Line	44	21.015	43
6	Lohmann	56	17.898	39
7	Lohmann	63	16.518	38
8	Lohmann	65	18.752	40
9	Lohmann	80	18.930	40
			TOTAL	362

RESULTS

Of the examined 362 fecal samples, 45 (12.4%) were found to be *Salmonella* positive. *Salmonella* isolation rates of each flock, Flock 1 to 9, were determined to be 0.00%, 9.52%, 0.00%, 14.63%, 23.25%, 10.25%, 13.15%, 17.5%, 22.50%, repectively (Table 2). *S. Enteritidis* and *S. Kentucky* were found to share the first place with the rate of 31.11%, followed by S. *Infantis* and *S. Mbandaka* with the rates of 26.66% and 11.11%, respectively which were found at second and third place among 45 *Salmonella* isolates.

The multi-serovar distribution among the flocks were as follows: Three *S. Infantis* and 1 *S. Enteritidis* in flock 2; 1 *S. Infantis* and 5 *S.* M*bandaka* in flock 4; 8 *S. Enteritidis* and 2 *S. Infantis* in flock 5; 2 *S. Infantis* and 5 *S. Kentucky* in flock 8; 5 *S. Enteritidis* and 4 *S. Infantis* in flock 9. Of the examined 9 flocks only two were found to be contaminated solely one

Table 2: Serovar distribution of *Salmonella* in commercial layer flocks

Flocks	Number of Serovars				Number of *Salmonella* spp. positive samples (%)	Number of collected fecal samples
	S. Enteritidis	*S. Infantis*	*S. Kentucky*	*S. Mbandaka*		
Flock1	–	–	–	–	–	38
Flock2	1	3	–	–	4 (9.52%)	42
Flock3	–	–	–	–	–	41
Flock4	–	1	–	5	6 (14.63%)	41
Flock5	8	2	–	–	10 (23.25%)	43
Flock6	–	–	4	–	4 (10.25%)	39
Flock7	–	–	5	–	5 (13.15%)	38
Flock8	–	2	5		7 (17.5%)	40
Flock9	5	4	–	–	9 (22.5%)	40
TOTAL	14 (31.11%)	12 (26.66%)	14 (31.11%)	5 (11.11%)	45 (12.4%)	362

serovar. Four and 5 *S. Kentucky* isolates were determined in flock 6 and flock 7, respectively (Table 2).

Approximately fifty-three percent (24/45 of *Salmonella* isolates) were found to be resistant to one or more of the antibiotics. Of the tested antibiotics, none of the isolates exhibited resistance to gentamycin (CN) and chloramphenical (C), while the highest resistance was found for tetracycline (TE) and the lowest Resistant was determined for ciprofloxacin (CIP) (Table 3).

In our study the percentage of 8.3, 25, 66.6, 66.6, 75, 75, and 83.3% resistance were determined in AMP, K, S, TRI, NA, SXT, and TE, respectively in *S. Infantis* isolates, the percentage of resistance to NA, TRI, SXT, and TE were found to be 28.5, 7.1, 7.1, and 7.1%, respectively for *S. Enteritidis* isolates. *S. Kentucky* isolates were determined to be resistant to AMP (21.4%), S (21.4%), NA (14.2%), CIP (7.1%), TE (21.4%). All the *S. Mbandaka* isolates were found to be resistant to K with the rate of 100% (Table 4).

All the *S. Infantis* isolates were found to be resistant to 2 or more than 3 antibiotics. Each of two *S. Infantis* from flocks 2 and 1 and from flock 9 were found to be resistant to NA/SXT/TE/W, respectively. NA/S/SXT/TE/W resistance were observed in 4 *S. Infantis* isolates, each of the 2 were isolated from flock 2 and flock 4; the remaining 2 *S. Infantis* isolates were from flock 9. In flock 5, one of 2 *S. Infantis* isolates exhibited K/NA/S/SXT/TE/W resistance profiles, the other were found to be resistant to AMP/K/NA/SXT/TE/W. S/TE. NA/SXT and K/S resistance profiles were observed in each S. *Infantis* isolates from flock 9 and flock 4, respectively.

As a result, all the *S. Infantis* isolates were multi-drug resistant (MDR), while 1 out of 14 *S. Enteritidis* was determined to be resistant to NA/SXT/TE/W from flock 5. Three out of 14 were found to be resistant to NA in flock 9 and the remaining 10 isolates were found to be sensitive to all the tested antibiotics. In flock 8, 1 *S. Kentucky* exhibited AMP/SE/TE resistance profile whereas AMP/CIP/NA/S/TE and AMP/NA/S/TE resistance profiles were observed in 2 *S. Kentucky* isolates from flock7, the remaining 11 *S. Kentucky* were found to be sensitive to all the tested antibiotics. All *S. Mbandaka* from flock 4 were determined to be resistant to K (Table 3).

DISCUSSION

The low rate of *Salmonella* spp. isolation from commercial layer flocks in the study of 12.4% was found to paralell studies reporting *Salmonella* detection rates as low as 0.0 to 17% from Turkey (11,16,17,18). A similiar low *Salmonella* prevalences in commercial layer flocks was also reported in United Kingdom (1.7%), France (17.9%), Japan (23.6%) and Bangladesh (18%) (9, 19, 20, 21). In a study conducted by Van Hoorebeke *et al.* (22), between flock prevalence of *Salmonella* was reported to be low in Switzerland (0.00%), Belgium (1.43%), Germany (20.00%) and Greece (20.00%). Contrary to these results, high *Salmonella* prevalences were reported in Kosova (49%), Canada (52%) and USA (86.5%) (7, 23, 24). Also, there have been some controversies regarding the high *Salmonella* isolation rates from Turkey reported by Carli *et al.* (10) and Temelli *et al.* (25) with an approximately 60% incidence in chicken layer flocks.

Table 3: Antibiotic resistance patterns of *Salmonella* serovars in commercial layer flocks

Flock	Isolates	AMP	C	CIP	CN	K	NA	S	SXT	TE	W
F2	SE	S	S	S	S	S	S	S	S	S	S
	SI	S	S	S	S	S	R	S	R	R	R
	SI	S	S	S	S	S	R	S	R	R	R
	SI	S	S	S	S	S	R	R	R	R	R
F4	SM	S	S	S	S	R	S	S	S	S	R
	SM	S	S	S	S	R	S	S	S	S	S
	SM	S	S	S	S	R	S	S	S	S	S
	SM	S	S	S	S	R	S	S	S	S	S
	SM	S	S	S	S	R	S	S	S	S	S
	SI	S	S	S	S	R	S	R	S	S	S
F5	SE	S	S	S	S	S	R	S	R	R	R
	SE	S	S	S	S	S	S	S	S	S	S
	SE	S	S	S	S	S	S	S	S	S	S
	SE	S	S	S	S	S	S	S	S	S	S
	SE	S	S	S	S	S	S	S	S	S	S
	SE	S	S	S	S	S	S	S	S	S	S
	SE	S	S	S	S	S	S	S	S	S	S
	SE	S	S	S	S	S	S	S	S	S	S
	SI	R	S	S	S	R	R	S	R	R	R
	SI	S	S	S	S	R	R	R	R	R	R
F6	SK	S	S	S	S	S	S	S	S	S	S
	SK	S	S	S	S	S	S	S	S	S	S
	SK	S	S	S	S	S	S	S	S	S	S
	SK	S	S	S	S	S	S	S	S	S	S
F7	SK	S	S	S	S	S	S	S	S	S	S
	SK	R	S	S	S	S	S	R	S	R	S
	SK	R	S	R	S	S	R	R	S	R	S
	SK	S	S	S	S	S	S	S	S	S	S
	SK	S	S	S	S	S	S	S	S	S	S
F8	SK	S	S	S	S	S	S	S	S	S	S
	SK	S	S	S	S	S	S	S	S	S	S
	SK	S	S	S	S	S	S	S	S	S	S
	SK	S	S	S	S	S	S	S	S	S	S
	SK	R	S	S	S	S	S	R	S	R	S
	SI	S	S	S	S	S	S	R	S	R	S
	SI	S	S	S	S	S	R	R	R	R	R
F9	SE	S	S	S	S	S	S	S	S	S	S
	SE	S	S	S	S	S	S	S	S	S	S
	SE	S	S	S	S	S	R	S	S	S	S
	SE	S	S	S	S	S	R	S	S	S	S
	SE	S	S	S	S	S	R	S	S	S	S
	SI	S	S	S	S	S	R	S	R	S	S
	SI	S	S	S	S	S	S	R	S	R	S
	SI	S	S	S	S	S	R	R	R	R	R
	SI	S	S	S	S	S	R	R	R	R	R

SE: *S. Enteritidis, SK: S. Kentucky, SI: S. Infantis, SM: S. Mbandaka*
AMP: Ampicillin; C: Chloramphenicol; CIP: Ciprofloxacin; CN: Gentamicin; K: Kanamycin; NA: Nalidixic acid; S: Streptomycin; SXT: Trimethoprim/sulfamethoxazole; TE: Tetracycline; W: Trimethoprim; R: Resistant; S: Susceptible

Table 4: Summary data on rate of antimicrobial resistance in *S. Enteritidis, S. Kentucky, S. Infantis* and *S. Mbandaka*

Serovars	Resistance to (%)									
	AMP	C	CIP	CN	K	NA	S	SXT	TE	W
S. Enteritidis	0.0	0.0	0.0	0.0	0.0	28.5	0.0	7.1	7.1	7.1
S. Kentucky	21.4	0.0	7.1	0.0	0.0	14.2	21.4	0.0	21.4	0.0
S. Infantis	8.3	0.0	0.0	0.0	25	75	66.6	75	83.3	66.6
S. Mbandaka	0.0	0.0	0.0	0.0	100	0.0	0.0	0.0	0.0	0.0

The serovar distribution and dominant *Salmonella* serovars varies from country to country. *S. Kentucky* and *S. Typhimurium* in USA with a 62% percentage (24) and 66.7% (26), respectively. S. *Heidelberg* in Canada with a 20% (7), *S. Enteritidis* in Turkey with a 70.1% (25), were reported to be the dominant serovars. In our study, both *S. Enteritidis* and *S. Kentucky* with 31.11% incidences were found to be dominant serovars. Subsequently, *S. Infantis* and *S. Mbandaka* were the other serovars with 26.66% and 11.11% incidences, respectively, circulating in commercial layer flocks, in Bandirma located in the Marmara Region of Turkey.

Of the 5 serovars given top priority by EU due to their public health concern are *S. Enteritidis*, *S. Typhimurium*, *S. Virchow*, *S. Infantis* and *S. Hadar*. Therefore, detection of presence of *S. Enteritidis* and *S. Infantis* in layer flocks was evaluated in greater depth.

The result of *S. Enteritidis* dominance in chicken layer flocks in the study concurred with the results of the other studies from Turkey conducted in Marmara Region (10, 25, 27). In addition a similiar dominance was reported from abroad compared to our study (9, 21, 22, 28). Combination of the results of the previous studies and our study points out that this pathogen is persistently present in chicken layer flocks in Turkey. Inappropriate hygiene and sanitation applications, ineffective and/or insufficient biosecurity, and insufficient vaccination applications make the flocks prone to the persistent infection with *S. Enteritidis.*

In the study, *S. Kentucky* was found to be the other dominant serovar in paralell to the studies conducted by Li *et al.* (24) and Andoh *et al.* (29). The spread and global persistence of *S. Kentucky* in different geographical regions and the spread of epidemic clones recovered from particularly poultry farms implicated *S. Kentucky* as a potential human infection vehicle (2). Hence, the dominance of *S. Kentucky* in the study cannot be ignored.

Among the serovars, the third place was determined to *S. Infantis* in our study. There have been other studies, which have reported *S. Infantis* in chicken layer flocks as a dominat serovar (20) from Japan as well as being detected in second place from Turkey (27). In many countries low to high prevealnce of S. *Infantis* in poultry farms has place emphasis of this bacteria as a public health concern (30, 31, 32).

The presence of *S. Mbandaka* has been reported in chicken layer flocks and poultry products throughout the world (8, 20, 33, 34, 35, 36). In the present study, the rate of *S. Mbandaka* in chicken layer flocks was not surprizing. Taken from a Polish study it should be taken into consideration that due to the close relationship between *S. Mbandaka* of human and poultry origin that animals may be a primary source of human infection (37).

Flock 2, 5, 9, and flocks 4 and flock 8 were contaminated with more than 1 serovar, combinations of *S. Enteritidis-Infantis*, *S. Infantis-Mbandaka*, S. *Infantis-Kentucky*, respectively. The multi-serovar contamination in chicken layer flocks was also reported by Lapuz *et al.* (38) and Im *et al.* (8).

In contrast to all *S. Infantis* determined to be sensitive to CIP in the study, according to the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) data (2014) based on laying hens, the percentage exhibiting resistance to CIP for *S. Infantis* isolates was observed at the highest rate (39). AMP resistance for *S. Infantis* isolates in the study coincided with the similar lowest rate with EFSA and EDC data (2014) (39). While the first similar highest percentage of resistance were observed in *S. Enteritidis* isolates against NA and CIP according to EFSA and EDC data (2014), in our study NA resistance for *S. Enteritidis* isolates have already been found highest, but not for CIP since all *S. Enteritidis* isolates were determined to be sensitive for CIP (39). In our study while NA resistance was found to be 14.2%, CIP resistance was determined as 7.1% for *S. Kentucky* according to EFSA and EDC, the percentage of CIP and NA resistance for *S.*

Kentucky isolates was reported to be 87.5% and 84.1, respectively (39).

The similarities and diversities between studies on the subject of antibiotics may be attributed to the difference in the frequency of flock exposure to antimicrobials. Resistance to antimicrobial drug can result from repeated abuse and therefore the high level of antimicrobial resistance of *Salmonella* isolates suggests an indiscriminate and continuous use of sub-therapeutic doses of such drugs in commercial layer flocks.

In our study, 1 *S. Enteritidis*, 3 *S. Kentucky*, and 12 *S. Infantis* isolates were found to be MDR. Parallel to our results, in a study conducted by Samanta *et al. (*40), a large majority of *S. Enteritidis* isolates were reported to be insensitive to antibiotics. When the resistance profile of MDR *S. Enteritidis* (NA/SXT/TE/W) was compared with the other studies, common resistance patterns shared by SXT/NA, SXT/TE, and SXT/TE/W with addditional microbials were determined (29, 41). In MDR *S. Kentucky* isolates, 3 different resistance profiles were observed. Resistance profile of AMP/CIP/NA/S/TE in one of the three coincided with the results of a study in poultry farms from Ghana (29). The author reported that all *S. Kentucky* isolates were resistant to more than 2 antibiotics and shared common resistance to NA/CIP/TE in combinations of AMP/CIP/NA/CN/SUL/TE and AMP/CIP/NA/SUL/TE (29). There were reports which corroborated the presence of MDR *S. Kentucky* sharing the same NA/CIP/TE pattern (18, 42, 43, 44). The resistance profile of *S. Kentucky* in the study was evaluated as a dissemination of bacterial clones showing specific resistance patterns because in Turkey, Poland and Ghana similiar specific resistance patterns were present (29, 45). All *S. Mbandaka* isolates were found to be resistant to K unlike the results of the studies Im *et al.* (8).

Consequently, high isolation rates of *Salmonella* serovars at commercial layer flocks provides useful information on the necessity of promptly taking effective and efficient preventive measures in commercial chicken layer flocks in Bandirma. An urgent need of programs specifically prepared for layer companies have to be applied promptly to combat against *Salmonella* serovars. Furthermore, antibiotic resistance patterns of the serovars, particularly MDR isolates, in chicken layer flocks in Bandirma was considered to be important for public health due to usage of common antibitiotics for treating humans. Transmission of resistant patterns from chicken to the public is a serious potential problem in the future.

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