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Short Communication

Prevalence, Antimicrobial Resistance and Molecular Characterization of *Salmonella* spp. and *Listeria monocytogenes* Isolated from Chicken Carcass

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Abstract

This study aimed to investigate the prevalence of *Salmonella* spp. and *Listeria monocytogenes,* their antimicrobial resistance profile. *L. monocytogenes* was not isolated from any of the samples. *Salmonella* spp. was detected from 32 (8%) out of the 400 collected samples. Antimicrobial resistance was most frequently observed to nalidixic acid (100%), tetracycline (93.75%), erythromycin (90.6%), streptomycin (84.3%), followed by kanamycin (62.5%). Also, 37.5% of *Salmonella* isolates were phenotypically confirmed as ESBL producers. Multiple drug resistance was defined 93.75% of the isolates. Among the *Salmonella* isolates, all of them harbouring *qnrB* and *qnrS* genes and, 37.5% of them presented bla_{TEM} gene..

Keywords: *Antimicrobial resistance, Carcass, Chicken, Listeria monocytogenes, Salmonella*

Tavuk Karkaslarından İzole Edilen *Salmonella* spp. ve *Listeria monocytogenes* Prevalansı, Antimikrobiyal Direnci ve Moleküler Karakterizasyonu

Öz

Bu çalışmada *Listeria monocytogenes* ve *Salmonella* spp. prevalansı ve antimikrobiyal direnç profillerinin araştırılması amaçlandı. *L. monocytogenes* örneklerden izole edilmedi. *Salmonella* spp. toplanan 400 örneğin 32 (%8)'sinden saptandı. Antimikrobiyal direnç en sık nalidiksik asit (%100), tetrasiklin (%93.75), eritromisin (%90.6), streptomisin (%84.3), ardından kanamisin (%62.5)'de belirlendi. Ayrıca, *Salmonella* izolatlarının %37.5'i ESBL pozitif olarak fenotipik yöntemlerle doğrulandı. Çoklu ilaç direnci, izolatların %93.75'inde tanımlandı. Salmonella izolatlarının hepsinin *qnrB* ve *qnrS* geni, %37.5'inin ise bla_{TEM} geni taşıdığı belirlendi.

Anahtar sözcükler: *Antimikrobiyal direnç, Karkas, Listeria monocytogenes, Salmonella, Tavuk*

INTRODUCTION

The poultry meat is one of the most favourite meat products being consumed worldwide. *Salmonella species and Listeria monocytogenes* are major foodborne pathogenic bacteria, and contaminants of raw poultry meat [1].

Salmonella species are Gram-negative, non-spore forming, non-lactose fermenting rod-shaped bacteria, and a member of the family Enterobacteriaceae. The genus Salmonella which is classified into two species, *Salmonella enterica* (type species) and *Salmonella bongori* based on differences in their 16S rRNA sequence analysis, includes more than 2600 different serotypes and most of these serotypes have the ability to adapt within a variety of animal hosts, including humans [1,2]. *L. monocytogenes* is gram-positive, rod-shaped, beta-hemolytic, motile, facultative intracellular bacteria, capable of surviving

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under refrigeration conditions, low pH and in high salt concentration [3].

The European Food Safety Authority (EFSA) reported 94.530 laboratory confirmed Salmonellosis cases in humans and *S. enteritidis and S. typhimurium* accounted for almost 80% of human cases acquired [1]. *S. enteritidis* and *S. typhimurium* are the most commonly reported serovars in the European Union (EU), being associated with 52.3% and 23.3% of all confirmed human salmonellosis, respectively [2]. With it, Salmonella was most frequently isolated in poultry, in 2016 [1]. The incidence of listeriosis is low worldwide, however, *L. monocytogenes* can cause severe and lethal infections, ranging from 20% to 30% (septicaemia, encephalitis and meningitis) during vulnerable periods of life (older adults, pregnant women and immunocompromised patients). Chicken products can be contaminated with *L. monocytogenes* during processing, or cross-contamination during preparation, cooking, and serving food for other foods [3].

Over recent decades, antibiotic resistance undoubtedly represents a global public health problem. Several reports and books have been published about antibiotic resistance problem and the reasons behind the increasing rates. It was highlighted that poultry meat may play a major role in transmission [4-6]. The inappropriate, uncontrolled and excessive use of antibiotics in the treatment of infections in humans and veterinary medicine may be the reason for high rates of resistance, in poultry [7].

The European Commission emphasize the requirement of co-ordinated research effort about antimicrobial resistance. With this aim in mind, the current study aimed to investigate the prevalence, antimicrobial resistance and molecular characterization of *Salmonella* spp. and *L. monocytogenes* in chicken carcass.

MATERIAL and METHODS

Sample Collection

A total of 400 chicken carcasses were collected from various retail markets in different districts of Istanbul (Ataşehir, Avcılar, Bakırköy, Başakşehir, Beşiktaş, Beylikdüzü, Beyoğlu, Eminönü, Fatih, Gaziosmanpaşa, Kadıköy, Kartal, Maltepe, Pendik, Şişli, Üsküdar) between July 2014 and December 2016. Fresh packaged chicken carcasses were transported to the laboratory under cold chain and analysed within 2 h.

Isolation and Species Identification

The detection of pathogens was performed following official methods: *Salmonella* spp. (ISO 6579:2002) [8] and *L. monocytogenes* (ISO 11290-2:2005) [9]. Suspected colonies were identified by API-20E for *Salmonella* spp. and by API-Listeria for *L. monocytogenes*. For confirmation and identification of the genus and species of the Salmonella isolates, multiplex PCR (mPCR) was performed [10]. Primers, band weight and references used are showed in *Table 1*.

Antimicrobial Susceptibility Testing

Isolates were tested for antibiotic susceptibilities by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) standards against 12 different antimicrobials in 7 antimicrobial classes, including those used to treat human listeriosis and salmonellosis: ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), erythromycin (15 µg), imipenem (10 µg), kanamycin (30 µg), meropenem (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), sulfamethoxazole/trimethoprim (1.25/23.75 µg) and tetracycline (30 µg).

Extended-spectrum beta-lactamases (ESBL) production was analysed by the double disk diffusion test containing cefotaxime and ceftazidime with and without clavulanic acid. An increase in the zone diameter of 5 mm or more when either of the antimicrobial agents was combined with clavulanic acid was considered evidence of ESBL production. The results were based on CLSI breakpoints [11].

Multiple drug resistance was defined as simultaneous resistance to clinically relevant drugs of at least three different classes. Moreover, the multiple antibiotic resistance (MAR) index was calculated using the formula: a/b, where 'a' represents the number of antibiotics to which a particular isolate was resistant and 'b' the total number of antibiotics tested, for all Salmonella isolates. As quality controls, *Escherichia coli* ATCC 25922 were tested in each run.

Detection of Antimicrobial Resistance Genes

All of the isolates were analysed for the presence of plasmid-mediated quinolone resistance (PMQR) genes and some of the genes encoding β-lactam resistance. The PMQR genes (*qnrA, qnrB, qnrS, qnrC, qnrD, qepA*, and *aac(6')-Ib-cr*) were

Table 1. *Primers and band weight used in the mPCR*

Bacteria	Gene	Primer Sequence (5' to 3', as synthesized)	Size (bp)
Salmonella spp.	*invA*	AAA CGT TGA AAA ACT GAG GA TCG TCA TTC CAT TAC CTA CC	199
S. Enteritidis	*sdf*	AAA TGT GTT TTA TCT GAT GCA AGA GG GTT CGT TCT TCT GGT ACT TAC GAT GAC	299
S. Typhimurium	STM4492	ACA GCT TGG CCT ACG CGA G AGC AAC CGT TCG GCC TGA C	759

detected with a polymerase chain reaction (PCR) assay using previously described primers and protocol [12]. Also, all phenotypically ESBL isolates were screened for genes encoding β-lactam resistance (*bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{OXA}$, and *bla*$_{CMY}$) using previously described methods [13,14].

RESULTS

Listeria monocytogenes was not isolated from any of the samples analysed. *Salmonella* spp. were recovered from 32 (8%) out of the 400 collected carcass samples by conventional culture technique. All of the Salmonella serovars were confirmed, and also, the predominant serovar was identified as *S. enteritidis* (71.8%, 23/32) followed by *S. typhimurium* (28.1%, 9/32) with mPCR assays.

The antimicrobial resistance was most frequently observed to nalidixic acid, tetracycline, and erythromycin, streptomycin, followed by kanamycin. No resistance to carbapenems, phenicol and sulphonamide was recorded *(Table 2)*. Moreover, 12 out of the 32 (37.5%) isolates were phenotypically confirmed ESBL producers and multiple drug resistance was defined 30 out of the 32 (93.75%) isolates. Antibiotic resistant profiles and multiple resistance index (MAR) of Salmonella isolates from chicken carcasses are presented in *Table 3*.

Table 2. *Antimicrobial susceptibility testing results of Salmonella isolates*

Classes		**Antimicrobial Agents**	**Number of Resistant Isolates (n=32)**	**Percentage of Resistance (%)**
Beta-Lactams	Penicillins	*Ampicillin*	4	12.5
	Cephalosporins	*Cefotaxime*	12	37.5
	Carbapenems	*Meropenem*	-	-
		Imipenem	-	-
Aminoglycosides		*Streptomycin*	27	84.3
		Kanamycin	20	62.5
Quinolones		*Nalidixic Acid*	32	100
		Ciprofloxacin	4	12.5
Tetracyclines		*Tetracycline*	30	93.75
Phenicols		*Chloramphenicol*	-	-
Macrolides		*Erythromycin*	29	90.6
Sulfonamides		*Sulfamethoxazole/Trimethoprim*	-	-

Table 3. *Antibiotic resistant profiles and multiple resistance index (MAR) of Salmonella isolates from chicken carcasses*

Isolates No	**Serovar**	**Resistance Phenotype Profile**	**Phenotypic ESBL Results**	**Genotypic PCR Results**	**MAR Index**
1, 10	*S.* Enteritidis	E, NA, TE	-	qnrB, qnrS	0.250
2, 23	*S.* Enteritidis	E, NA	-	qnrB, qnrS	0.166
3, 4, 5, 13, 14, 15	*S.* Enteritidis	CFX, E, K, NA, S, TE	+	*bla*$_{TEM}$, *qnrB, qnrS*	0.500
6	*S.* Enteritidis	K, NA, S, TE	-	qnrB, qnrS	0.333
7, 17	*S.* Enteritidis	AMP, CFX, E, K, NA, S, TE	+	*bla*$_{TEM}$, *qnrB, qnrS*	0.583
8, 16, 18	*S.* Enteritidis	E, K, NA, S, TE	-	qnrB, qnrS	0.416
9, 11, 12, 19, 20, 22	*S.* Enteritidis	E, NA, S, TE	-	qnrB, qnrS	0.333
21	*S.* Enteritidis	CIP, NA, TE	-	qnrB, qnrS	0.250
24	*S.* Typhimurium	CFX, K, NA, S, TE	+	*bla*$_{TEM}$, *qnrB, qnrS*	0.416
25	*S.* Typhimurium	K, NA, S, TE	-	qnrB, qnrS	0.333
26	*S.* Typhimurium	AMP, CFX, CIP, K, NA, S, TE	+	*bla*$_{TEM}$, *qnrB, qnrS*	0.583
27, 29, 32	*S.* Typhimurium	E, K, NA, S, TE	-	qnrB, qnrS	0.416
28	*S.* Typhimurium	CFX, CIP, K, NA, S, TE	+	*bla*$_{TEM}$, *qnrB, qnrS*	0.500
30	*S.* Typhimurium	AMP, CFX, E, K, NA, S, TE	+	*bla*$_{TEM}$, *qnrB, qnrS*	0.583
31	*S.* Typhimurium	CIP, NA, S, TE	-	qnrB, qnrS	0.333
Average: 0.400					

AMP, Ampicillin; CFX, Cefotaxime; CIP, Ciprofloxacin; E, Erythromycin; K, Kanamycin; NA, Nalidixic Acid; S, Streptomycin; TE, Tetracycline

All of the *Salmonella* isolates were positive for the presence of *qnrS* and *qnrB* genes. None of the tested isolates carried *qnrA, qnrC, qnrD*, *qepA,* or *aac(6')-Ib-cr.* Also, 12 out of the 32 (37.5%) isolates carried only bla_{TEM} genes. None of the isolates presented bla_{CTX-M}, bla_{SHV}, bla_{OXA}, and bla_{CMY} genes.

DISCUSSION

European Food Safety Authority [1] accounted 2.536 laboratory confirmed Listeriosis cases in humans and also *L. monocytogenes* prevalence reported as 0.8% from broiler meat samples. Several reports have highlighted that poultry meat may play a major role in transmission [1,3]. In the current study, *L. monocytogenes* was not isolated from any of the samples. This promising result might be the consequences of applied biosecurity measures.

Worldwide, various prevalence rates of *Salmonella* spp. ranged from 0% to 100% was reported in poultry [2,4,5]. The authors emphasize that differences of Salmonella prevalence in chicken meat could be based on the geographical differences, sampling techniques, detection methods, slaughterhouse hygiene and cross-contamination of products [5,6]. Regarding previous studies in Turkey, *Salmonella* spp. was reported in 34% of packaged fresh raw chicken samples using cultural technique and PCR [4]. When comparing the reported prevalence among our country during recent years, the results of the present study are considerably lower. In addition, the authors reported that *S. typhimurium* was predominant ones recovered from chicken samples [4]. Contrary, in the presented study, *S. enteritidis* was predominant, followed by *S. typhimurium*.

The emergence of antimicrobial resistance is not a new phenomenon, nor an unexpected one. Nowadays, incidence of antimicrobial resistance in *Salmonella* spp. isolated from especially poultry products, has increased. In the current study, 93.75% of the Salmonella isolates was classified as multidrug resistant. Our results were relatively high according to some studies [15,16] while showed similarities to others [4,6].

In the current study, all of the *Salmonella* isolates were resistant to nalidixic acid, and most of them to tetracycline, erythromycin, streptomycin and kanamycin. These results substantiate other authors' findings [1,2,6]. The broad use of these classes of antibiotics in poultry may be the reason for this crucial problem.

Plasmid-Mediated Quinolone Resistance determinants are widely distributed among *Enterobacteriaceae*, including *Salmonella*, worldwide. Regarding previous studies, the most commonly identified resistance determinants are *qnrA, qnrB, qnrS,* and *aac(6')-Ib-cr* genes, in Turkey [17]. In the present study, PMQR mechanisms in all of the Salmonella isolates were identified as *qnrS* and *qnrB*. None of the tested isolates carried *qnrA, qnrC, qnrD*, or *qepA, aac(6')-Ib-cr.*

Plasmid-Mediated Quinolone Resistance determinants are often combined with extended-spectrum beta-lactamases (ESBLs) which are less prevalent in Salmonella. Prevalence of ESBL-producing *Salmonella* species isolated from humans and animals has been reported in many parts of the world [5,18]. On the contrary, foodborne ESBL-producing Salmonella has been rarely published. In the current study, 12 of the 32 (37.5%) isolates showed resistance against extended spectrum cephalosporin's phenotypically and carried only bla_{TEM} genes, which have been stated to be one of the most widely distributed β-lactamase. These results might be consequences of the study limits, only some of the resistance determinants studied, so any another resistant gene could not be determined.

Globally, the author's highlight that poultry meat is a potential hazard for public health and the essential precautions should be taken to ensure improving the quality. In the current study provides baseline information on the highlights the widespread presence of the emerging foodborne pathogens and resistance profiles in poultry meat. Further multidisciplinary studies and novel strategies in the spirit of 'One Health' are needed.

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