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PREVALENCE AND ANTIBIOTIC RESISTANCE OF *SALMONELLA* SPP. AND *SALMONELLA* TYPHIMURIUM IN BROILER CARCASSES WINGS AND LIVER

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ABSTRACT

The objectives of this study were to isolate and identify *Salmonella* spp. from broiler carcasses, wings and liver samples by immunomagnetic separation based cultivation technique, to verify the isolates as *Salmonella* spp. by the detection of *oriC* gene by PCR, to identify the isolates using malic acid dehydrogenase and DT104 specific primers as *S.* Typhimurium and *S.* Typhimurium DT104. Also to determine the two important virulence genes, virulence plasmid (*spvC*) and invasion (*invA*) for molecular characterization, to evaluate the antibiotic resistance profiles of the isolates. For this purpose, 110 broiler carcasses, 110 broiler wings and 110 broiler liver samples with a total number of 330 were analyzed. Ninety six (29.1%) of the samples were detected as contaminated with *Salmonella* spp. According to the results 11 isolates (11.4%) were identified as *S.* Typhimurium. None of these serotypes were determined as specific phage type DT104. *InvA* gene was detected from all the (100.0%) *Salmonella* isolates and 14 isolates (14.6%) were detected as positive for *spvC* gene. Eighty-three isolates (86.4%) were resistant to at least 5, 70 isolates (72.9 %) resistant to at least 7 and 36 isolates (37.5%) were resistant to at least 9 antibiotic.

PRACTICAL APPLICATION

This work is significant because *Salmonella* is still an important public health problem all around the world. This study would provide some data about the incidence of *S.* Typhimurium and *S.* Typhimurium DT104 in chicken meat and parts and the antibiotic resistance of the isolates in Turkey. Besides, the method used in study and the parts chosen for analysis would be a model for the other researchers who are thinking to study in this area.

INTRODUCTION

Salmonella is one of the most common foodborne pathogen and causes important health risks and economic problems throughout the world. This bacterium has a major public health role which can infect people by consumption of different foods (Jorgensen *et al.* 2002). Non-typhoidal *Salmonella* spp. are considered as zoonotic agents and foods are the main sources for transmission to people. Various foods can be contaminated by *Salmonella* via cross contamination and also water can be a source as well (Hendriksen *et al.* 2011; Mukhopadhyay and Ramaswamy 2012). Among all other animal originated foods, chicken meat remains important as the most risky food to human health. Especially, *Salmonella enterica* subspecies *enterica* has so many serotypes which can be pathogenic for humans and other animals (D'Aoust and Maurer 2007). EFSA (European Food Safety Authority) surveillance data shows that some further contamination occur during slaughtering of broiler. According to the studies, the intestines of 71% of live chickens have already contaminated with *Salmonella* spp. while the ratio raise to 76% of sampled carcasses (EFSA 2014).

The majority of human cases of non-typhoidal salmonellosis are caused by a limited number of serovars, which may vary from country to country and over time (Hendriksen *et al.* 2011). *Salmonella* spp. gastroenteritis is generally a self-limiting illness and antibiotics may be required in some patients, particularly in vulnerable groups such as young children, the elderly and the immunocompromised people (Galanakis *et al.* 2007). Outbreak data and case–control studies have linked some serotypes to certain foods or exposures (Braden 2006). Information obtained during outbreak investigations is for understanding which foods are common sources of pathogens contributing to foodborne infections. During outbreak investigations, illnesses can be linked to a particular food by using epidemiologic or laboratory evidence (Jackson *et al.* 2013). As the most important source of *Salmonella,* chicken meat and products have high risk potential for human health and various serotypes can be detected by using different methods. At the same time, antimicrobial resistance has to be considered as a major public health problem for several food pathogens as well as *Salmonella* spp. Antibiotic resistance has to be determined from either foods or human origin *Salmonella* isolates (WHO 2005). Chicken meat and some edible offals could be contaminated with *Salmonella* in different production steps as well (in slaughterhouses, during processing, etc.) (Harker *et al.* 2014).

In this study, the aims were to isolate and identify *Salmonella* spp. from packaged broiler carcasses, wings and liver samples which were collected from Ankara markets by immunomagnetic separation (IMS) based cultivation technique. Also verifying the isolates as *Salmonella* spp. by the detection of *oriC* gene by PCR (polymerase chain reaction), to identify the isolates using malic acid dehydrogenase and DT104 specific primers as *S.* Typhimurium and *S.* Typhimurium DT104, respectively. Also to determine the two important virulence genes including, virulence plasmid and invasion for molecular characterization, to evaluate the antibiotic resistance profiles of the isolates with 20 different antibiotics.

MATERIAL AND METHODS

Sample Design and Collection

A total of 110 packaged broiler carcasses, 110 packaged broiler wings and 110 packaged broiler liver samples were obtained from different markets in Ankara between January 2009 and March 2012. The samples were taken into laboratory in an ice bag and analyzed within 2 h.

Isolation and Identification of *Salmonella* spp.

In the study, conventional cultivation and IMS based cultivation techniques were used for the isolation of *Salmonella* from broiler carcasses, wings and liver samples.

Immunomagnetic separation Based Cultivation Method

ISO 6579 technique was used for the isolation of *Salmonella* (Anon 2002). Twenty-five grams of broiler wing and liver samples was weighted separately in sterile bags and enriched with 225 mL buffered peptone water (BPW, Oxoid CM1049) and incubated at 37C for 24 h. For broiler carcass samples, rinse method was used and enriched with 500 mL BPW at 37C for 24 h. After the incubation period, IMS was performed with 20 μL of magnetic beads coated with specific antibody against *Salmonella* (Dynabeads anti-*Salmonella*, Prod. No. 710.02, Dynal, Oslo, Norway) according to the manufacturer's protocol (Anon 1991). Then, 100 μL of resuspended IMS mixture was plated on Brilliant-green Phenol-red Lactose Sucrose Agar (BPLS, Merck 7236, Hohenbrunn, Germany) and Xylose-Lysine Desoxycholate Agar (XLD, Oxoid CM0469) and incubated at 37C for 24–48 h. Typical grown colonies (up to five different colonies) were picked and inoculated into Triple Sugar Iron Agar (TSIA, Oxoid CM0277), Lysine Iron Agar (LIA, Oxoid CM0381) and Urea Broth Base (Oxoid CM0071B) and incubated at 37C for 24–48 h. TSIA positive, LIA positive and urease negative colonies were considered as suspect *Salmonella* spp. colonies. The agglutination test was done with polyvalent *Salmonella* Antisera (Difco, Cat. No L840114-1, Detroit, MI). Each suspect *Salmonella* colony was mixed with a drop of antiserum on a slide. Agglutination with antiserum was accepted as a positive reaction for *Salmonella* spp.

DNA Extraction

Isolates that stored at 4C in Tryptone Soy Agar (TSA, Oxoid CM 131) were incubated in Brain Heart Infusion broth (BHI, Oxoid CM0225) at 37C for 24 h. Then 1 mL of each enrichment culture was centrifuged (Eppendorf Centrifuge 5417R, Hamburg, Germany) for 15 min at 5,000 rcf at 10C. The pellets were resuspended in 1 mL sterile aquabidest. The suspensions were mixed by vortex (IKA MS1 Minishaker, Wilmington, DE). Then all tubes were centrifuged for 5 min at 5,000 rcf at 10C. The pellets were resuspended with 200 μL sterile aquabidest and incubated for 20 min at 95C in a water bath (Memmert WB/OB 7-45, WBU 45, Schwabach, Germany) and then cooled on ice.

PCR Analysis for the Detection of *oriC* Gene

OriC gene specific primers (primer 1: 5′-TTA TTA GGA TCG CGC CAG GC-3′; primer 2: 5′-AAA GAA TAA CCG TTG TTC AC-3′) (Promega, Madison, WI) that produce a 163 bp DNA fragment were used for the verification of the *Salmonella* isolates. PCR was performed with a final volume of 50 μL reaction mixture containing incomplete 1 × PCR Buffer (Promega M7921, Madison, WI), 1.5 mM $MgCl_2$, 200 mM each of the deoxynucleoside triphosphates, 1 U Taq

DNA polymerase, 0.50 mM each of primer and 10 μL DNA. Thermal cycling (Biometra Personal Cycler, Goettingen, Germany) was carried out with the initial denaturation at 94C for 1 min and then 35 cycles of denaturation at 94C for 1 min, annealing at 53C for 1 min, and extension at 72C for 1 min, 72C, 10 min for final extension (Widjojoatmodjo *et al.* 1991; Fluit *et al.* 1993; Erol *et al.* 1999).

PCR Analysis for *mdh* Gene and DT 104 Genes

PCR analysis was used in order to determine *S.* Typhimurium. The PCR was performed with *S.* Typhimurium specific gene *mdh* (malic acide dehyrogenase) primers (*mdh* primer F: TGC CAA CGG AAG TTG AAG TG; *mdh* primer R: CGC ATT CCA CCA CGC CCT TC) (Promega) in a final volume of 15 μL containing 1 × Reaction Buffer, 1.25 mM $MgCl_2$, 0.1 mM dNTPs each, 0.1 μM primers each, 0.75 U *Taq* DNA polymerase and 5 μL template DNA. Thermal cycling (Biometra, Personal cycler) was carried out with initial denaturation at 95C for 12 min and followed by 35 cycles of denaturation at 94C for 20 s, annealing at 68C for 30 s, extension at 72C for 40 s with a final extension at 72C for 4 min (Leon-Velarde *et al.* 2004). All *S.* Typhimurium positive isolates were controlled with PCR protocol for DT104 specific gene (DT104 primer F: GTC AGC AGT GTA TGG AGC GA; DT104 primer R: AGT AGC GCC AGG ACT CGT TA). DT 104 protocol's PCR mix contains 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.0 mM $MgCl_2$, 200 μM dNTPs each, 0.10 μM primers each and 1.25 U *Taq* DNA polymerase and 5 μL template DNA for 15 μL final volume. Thermal cycling (Biometra, Personal cycler) was carried out with initial denaturation at 96C for 1 min and followed by 30 cycles of denaturation at 96C for 30 s, annealing at 60C for 30 s, extension at 72C for 35 s, with a final extension at 72C for 30 s (Pritchett *et al.* 2000).

PCR Analysis for *Salmonella* Virulence Genes (*spvC* and *invA* Genes)

For this purpose, PCR procedure was performed according to Chiu *et al.* (2006) and Pritchett *et al.* (2000) by using primers specific for virulence plasmid (*spvC*) and invasion (*invA*) genes, respectively (Table 1). PCR reaction for *spvC* of 50 μL consisted of 1 × PCR buffer (20 mMTris-HCl (pH 8.4), 50 mM KCl), 1.5 mM $MgCl_2$, 200 μM dNTP each, 1 U Taq DNA polymerase, 2 μL template DNA and 0.1 μM of each primer pairs. After an initial denaturation at 95C for 1 min, thermal cycler (Biometra, Personal cycler) conditions were as follows: 35 cycles of denaturation at 95C for 45 s, annealing at 56C for 45 s, extension at 72C for 1 min with a final extension at 72C for 5 min. For *invA* gene, PCR protocol mix contains 2.0 mM $MgCl_2$, 200 μM dNTP each, 1.25 U Taq DNA polymerase, 5 μL template DNA and 20 mMTris-HCl (pH 8.4), 50 mM KCl for 25 μL final volume. For amplification, 30 cycles performed by Thermal Cycler (Biometra, Personal cycler). Initial denaturation at 96C for 1 min, and followed by 30 cycles of denaturation at 96C for 30 s, annealing at 60C for 30 s, extension at 72C for 35 s with a final extension at 72C for 30 s. For all PCR analyzes *S.* Typhimurium ATCC 14028 and *S.* Typhimurium DT104 ATCC 700408 were used as positive controls in this study.

TABLE 1. PRIMERS USED IN THE PCR ASSAYS

Primers	Sequence (5′–3′)	Target gene	PCR products (bp)
*ori*C-F	TTATTAGGATCGCGCCAGGC	*oriC*	163
*ori*C-R	AAAGAATAACCGTTGTTCAC		
mdh-F	TGCCAACGGAAGTTGAAGTG	*mdh*	216
mdh-R	CGCATTCCACCACGCCCTTC		
DT104-F	GTCAGCAGTGTATGGAGCGA		162
DT104-R	AGTAGCGCCAGGACTCGTTA		
*inv*A-1	ACAGTGCTCGTTTACGACCTGAAT	*invA*	
*inv*A-2	AGACGACTGGTACTGATCGATAAT		243
*spv*C-1	ACTCCTTGCACAACCAAATGCGGA	*spvC*	447
*spv*C-2	TGTCTCTGCATTTCGCCACCATCA		

Gel Electrophoresis

A 10 μL aliquot of each resultant PCR product was further analyzed by agarose gel (SeaKem LE Agarose, Rockland, ME) electrophoresis (Biometra, Agagel-Maxi-System B15359), stained with 0.1 μg/mL ethidium bromide (Merck 111608), at 100 V for 1 h and visualized by a gel documentation and analysis system (Sygene Ingenius, Cambridge, UK) (Goncuoglu *et al.* 2010).

Antimicrobial Susceptibility Tests

The antibiotic susceptibility test of *Salmonella* isolates was performed with the disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) (Anon 2006) in Mueller-Hinton agar (Oxoid CM0337) with 20 different antibiotics as shown in Table 2. *Salmonella* isolates were transferred to BHI broth (Oxoid CM0225) and incubated at 37C for 24 h. A loopfull of growth was transferred into 5 mL containing Tryptone Soya Broth (Oxoid CM0129). The culture was incubated at 37C until it achieved the turbidity of the 0.5 McFarland standard. The correct density of the turbidity standard was measured by NanoDrop Spectrophotometer (NanoDrop ND-100, DE). The absorbance of the broth cultures at 625 nm was fixed at 0.08–0.10. The suspension was inoculated uniformly to Mueller Hinton agar (uniform depth of 4 mm) with a sterile cotton swab. Then, antibiotic discs were placed onto the plate and incubated at 37C for 18 h. According to the sizes of the inhibition zones, interpretation of the strains as susceptible, intermediate or resistant was made according to the CLSI.

TABLE 2. ANTIBIOTIC RESISTANCE PROFILES OF *SALMONELLA* ISOLATES

	Salmonella positive isolates (%)		
Antibiotics (μg/disc)	R*	I*	S*
Ampicillin (AMP-10)	35 (36.45)	2 (2.08)	59 (61.45)
Cephazolin (KZ-30)	28 (29.16)	40 (41.66)	28 (29.16)
Cephalothin (KF-30)	23 (23.95)	31 (32.29)	42 (43.75)
Gentamicin (CN-120)	6 (6.25)	3 (3.12)	87 (90.62)
Amikacin (AK-30)	8 (8.33)	47 (48.95)	41 (42.70)
Amoxicillin/clavulanic acid (AMC-30)	7 (7.29)	9 (9.37)	80 (83.33)
Cefoxitin (FOX-30)	3 (3.12)	22 (22.91)	71 (73.95)
Ceftriaxone (CRO-30)	–	6 (6.25)	90 (93.75)
Ciprofloxacin (CIP-5)	6 (6.25)	41 (42.70)	49 (51.04)
Imipenem (IPM-10)	–	–	96 (100.00)
Trimethoprim/sulphamethoxazole (SXT-25)	71 (73.95)	2 (2.08)	23 (23.95)
Chloramphenicol (C-30)	31 (32.29)	48 (50.00)	17 (17.70)
Kanamycin (K-30)	24 (25.00)	55 (57.29)	17 (17.70)
Tetracycline (TE-30)	93 (96.87)	2 (2.08)	1 (1.04)
Trimethoprim (W-5)	74 (77.08)	17 (17.70)	5 (5.20)
Sulphonamide compounds (S3-300)	95 (98.95)	–	1 (1.04)
Ceftiofur (EFT-30)	–	–	96 (100.00)
Streptomycin (S-10)	74 (77.08)	21 (21.87)	1 (1.04)
Nalidixic acid (NA-30)	73 (76.04)	18 (18.75)	5 (5.20)
Sulphamethoxazole (RL-25)	96 (100.00)	–	–

* R: Resistant, I: Intermediate, S: Susceptible

RESULTS

According to IMS technique based cultivation, 96 (29.1 %) of 330 samples were found to be contaminated with *Salmonella* spp. Within them *Salmonella* was detected from 28 (25.4 %) out of 110 broiler carcasses, 31 (28.1 %) out of 110 broiler wings and 37 (33.6 %) out of 110 broiler liver samples. All *Salmonella* spp. isolates were confirmed by detection of *oriC* gene (Fig. 1). According to the serotyping results 11 (11.4 %) of 96 total isolates were confirmed as *S.* Typhimurium positive (Fig. 2). Eight (72.2 %) and three (27.2 %) of them were recovered from liver and wing samples, respectively. None of 11 *S.* Typhimurium isolates were detected as DT 104 phage type. All isolates were also analyzed for *inv*A and *spv*C genes which are considered as important virulence genes for *Salmonella*, and 14 (14.6 %) of 96 positive isolates had *spv*C gene and all of 96 (100 %) isolates had *inv*A gene.

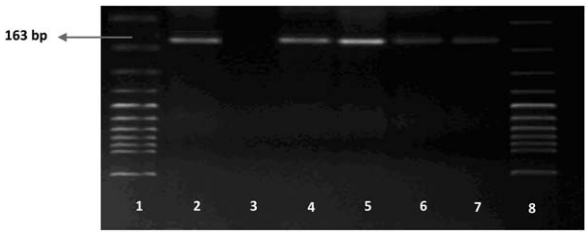

FIG. 1. *ORI*C GENE DETECTED *SALMONELLA* SPP. ISOLATES BY PCR. (1 AND 8: 100 BP DNA MARKER, 2: POSITIVE CONTROL – *S.* TYPHIMURIUM ATCC 14028, 3: NEGATIVE CONTROL, 4–7: *ORI*C POSITIVE *SALMONELLA* SPP. ISOLATES)

In this study, all *Salmonella* isolates were resistant to sulfamethoxazole and susceptible to ceftiofur and imipenem. High resistance profiles were found for sulfonamide compounds (98.9 %) and tetracycline (96.9 %), while high rates were detected for streptomycin, trimethoprim, nalidixic acid and trimethoprim/sulfamethoxazole with a ratio of 77.1, 77.1, 76.0 and 74.0%, respectively. Among 96 *Salmonella* isolates 83 (86.4%) of them were resistant to at least five, 70 (72.9%) of 96 isolates were resistant to at least seven and 36 (37.5%) of 96 isolate were resistant to at least nine antibiotics. Among *S.* Typhimurium, 9/11 (81.8%), 8/11 (72.7%) and 5/11 (45.4%) isolates were resistant to > 5, > 7 and > 9 antimicrobial , respectively. Within them all of them were resistant to tetracycline and 10 of them (90.9 %) to sulfonamide compounds (Table 2).

DISCUSSION

The present study demonstrated that broiler carcasses, wings and liver samples were quite contaminated with *Salmonella* spp. in Turkey with a contamination level of 29.1%. According to the previous studies conducted in all over the world, general contamination level of poultry meat was between 2 and 60% (Plummer *et al.* 1995; Rusul *et al.* 1996; Uyttendaele *et al.* 1998; Beli *et al.* 2001; Harrison *et al.* 2001; Zhao *et al.* 2001; Dominguez *et al.* 2002; Antunes *et al.* 2003; Jordan *et al.* 2006; Capita *et al.* 2007; Alalı *et al.* 2012; Donado-Godoy *et al.* 2012; Ta *et al.* 2014). In EU countries, *Salmonella* in fresh broiler meat at retail level was between 0

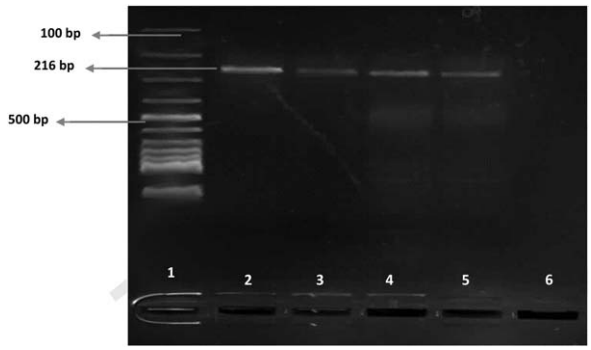

FIG. 2. *MDH* GENE DETECTED *SALMONELLA* SPP. ISOLATES BY PCR. (1: 100 BP DNA MARKER, 2: POSITIVE CONTROL – *S*. TYPHIMURIUM ATCC 14028, 3–5: *MDH* POSITIVE *SALMONELLA* SPP. ISOLATES, 6: NEGATIVE CONTROL)

and 33.3% according to 2013 data (Anon 2015). In Canada, 295 of 995 raw chicken meat was reported as *Salmonella* positive (Parmley *et al*. 2013). In the USA within all *Salmonella* outbreaks, 30% of them were associated with poultry meat. USDA FSIS (United States Department of Agriculture Food Safety and Inspection Service) data showed that 8.3% of broiler carcasses and 22.9% of ground chicken meat was contaminated with *Salmonella* (USDA 2015).

In a study conducted in Turkey, similar to our results, Mutluer *et al*. (1992) reported that 27.5% of broiler carcasses and broiler meat parts were contaminated with *Salmonella* spp. On the other hand, according to their serotyping results, 25.5% of their isolates were detected as *S*. Typhimurium. In another study, from 400 broiler carcass samples only 9.3% of the isolates were serotyped as *S*. Typhimurium (Dogru *et al*. 2010). Also, lower *S*. Typhimurium contamination rate (1.4%) had been reported in broiler carcasses and edible offal samples in Turkey (Erol *et al*. 2010). In this study, the contamination rate of wing (28.1%) and liver (33.6%) samples were found to be higher than carcass (25.4%) samples. Higher rates from carcass and wing samples may be due to cross-contamination in evisceration process in slaughterhouses. As liver had been reported as positive for *Salmonella* even one year of inoculation of one-day chicks, our high contamination rate in liver samples can be explained by this fact (Skov *et al*. 2002). Due to common consumption of chicken liver in Turkey, high *Salmonella* contamination rate, particularly for *S*. Typhimurium, which has obtained from this study, pointed out an important public health problem.

Although *Salmonella* contamination rates in broiler meat and parts are in a wide range, as written above, overall results of this study were in correlation with these previous data. This wide variation in *Salmonella* contamination in broiler meat could be linked to geographical, studying period, sampling techniques and analyzing method differences. Also general slaughterhouse processing differences, hygienic practices and contamination which occurred after slaughter and/or retail could be remarked as the main reasons of this variation.

As expected, all *Salmonella* isolates (100%) were carrying *invA* gene. From 96 *Salmonella*, 14 of them were positive for *spvC* gene (14.6 %) that codes virulence plasmid which plays an important role in virulence, survival and growth in host cells (Finlay and Falkow 1989). Similar to our results Swamy *et al*. (1996) have found that all *Salmonella* ($n = 245$) were positive for *invA* and 15.1% were positive for *spvC* genes isolated from poultry products, wastewater and human sources. On the other hand, Mohamed *et al*. (2014) studied molecular characterization and antibiotic resistance of both *S*. Typhimurium and *S*. Kentucky isolated from pre- and post-chilled broiler carcasses and found that only 1.3% of the isolates were carrying *spvC* gene and all of them were identified as *S*. Typhimurium. In our study from 14 *spvC* positive *Salmonella*, only two of them were *S*. Typhimurium (two different liver samples). Diarra *et al*. (2014) reported that 25.9% of their isolates were carrying *spvC* gene within them *S*. Typhimurium and *S*. Enteritidis were positive at a rate of 71.7% and 65.4%, respectively. These results showed the importance of serotyping and more researches are needed to be done for understanding the mechanism of *spvC* gene and virulence plasmid of *Salmonella*.

According to antibiogram results, *Salmonella* isolates were determined as high and/or intermediate resistant to 20 antibiotics tested in this study (Table 2).

As the antibiotic resistance profiles were considered, 83 isolates (86.45%) were resistant to 5 and more, 70 isolates (72.91%) resistant to 7 and more, 36 isolates (37.50%) were resistant to 9 and more antibiotic. Resistance to the large number of antibiotics was found in the other studies. The percentage of multi-resistant *S. enterica* strains was observed to be 100% by Álvarez-Fernández *et al*. (2012). Yang *et al*. (2010) reported that ∼28% of chicken isolates were resistant to 9 or more antimicrobials. Parveen *et al*. (2007) detected resistance to 8 antimicrobials in two *Salmonella* isolates from poultry. Shrestha *et al*. (2010) reported that high percentages of *Salmonella* isolates from poultry in Nepal were resistant to 5 (15.4%), 6 (69.2%), 7 (7.7%) and 8 (5.2%) antimicrobials. Pan *et al*. (2010) isolated two *S*. Pullorum strains resistant to 12 antimicrobials in China. In Korea, Hur *et al*. (2010) reported a *S*. Enteritidis strain isolated from poultry resistant to 15 antimicrobials. These findings confirm that poultry is an important reservoir of multi-resistant *Salmonella*, and suggest it is difficult to achieve successful antimicrobial therapy for salmonellosis caused by strains of poultry origin.

In the present study, all *Salmonella* isolates were resistant to sulfamethoxazole and susceptible to ceftiofur and imipenem. Susceptibility to beta-lactam antibiotics is a positive situation, however increase in the development of sulfamethoxazole

resistance is extremely worrisome, because of resistance to therapeutically important antimicrobial agents is becoming a serious concern.

One of the most important findings of this study is high intermediate resistance profiles of the isolates to most of the tested antibiotics. More than 30% of the *Salmonella* isolates were intermediately resistant to kanamycin, chloramphenicol, ciprofloxacin, amikacin, cephazolin, cephalothin (Table 2). When these results were compared with the previous findings, emergence of antibiotic resistance has been increasing year by year.

The increase in resistance to tetracycline and enrofloxacin observed in the study by Capita *et al.* (2007), study between 1993 and 2006, is not surprising because these drugs have been among the antibiotics most frequently used therapeutically on poultry farms in Spain. These findings confirm that therapeutic uses of antibiotics in animals are a serious health concern. In our study, higher resistance was found against sulfonamide compounds at 98.9% and tetracycline at 96.9%, too.

This study showed that there is an increase in the resistance to streptomycin, trimethoprim, nalidixic acid and trimethoprim/sulfamethoxazole with a ratio of 77.1%, 77.1%, 76.0% and 74.0%, respectively. Especially nalidixic acid findings are important for the use of quinolones. There is a special concern in the emergence of resistance to quinolones, fluoroquinolones or extended-spectrum cephalosporins such as ceftiofur and ceftriaxone. Recently, the occurrence of the resistance to these antibiotics in *Salmonella* isolates has increased. Therefore, continuous monitoring of its prevalence and resistance in the food supply is necessary because of the public health implications of a potential spread of resistant microorganisms.

Quinolones and third generation cephalosporins have been the antibiotics of choice in treating infections with MDR (Multi Drug Resistance) *Salmonella* (Karon *et al.* 2007). However, the emergence of *Salmonella* serotypes resistant to quinolones and cephalosporin poses a new challenge in treating infected patients, and the lack of an effective antibiotic therapy may lead to an increase in the morbidity and mortality rates.

Fluoroquinolones were approved in numerous European countries from the 1980s onwards. In the decades following the licensing of these drugs, there has been an increasing prevalence of quinolone resistant *Salmonella*, observed in clinical and poultry isolates both in Spain (Jiménez-Sáenz *et al.* 2001) and worldwide (Schroeter *et al.* 2004; Wilson 2004; de Oliveira *et al.* 2005; Ellerbroek *et al.* 2010; Shrestha *et al.* 2010). Fluoroquinolones are effective for treating a variety of clinical and veterinary infections including salmonellosis, urinary tract infections, gastrointestinal infections and respiratory tract infections (Reid 1992; Chen and Lo 2003).

In conclusion, our data confirm that chicken carcasses, wings and liver may be vehicles for transmitting *Salmonella*. From the isolates, some are carrying a *spvC* gene which plays an important role in virulence, survival and growth in host cells. These results showed the importance of more research needed for understanding mechanism of and virulence plasmid of *Salmonella*. *Salmonella* isolates were determined as high and/or intermediate resistant to 20 antibiotics tested in this study. As a result, continuous monitoring of *Salmonella* prevalence and resistance in the food supply is necessary because of the public health implications of a potential spread of resistant microorganisms.

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