Prevalence and Characterization of *Salmonella* Isolated from Chicken Meat in Turkey

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Abstract: This study was conducted in a Turkish province to investigate the presence of *Salmonella* spp. in 150 chicken meat samples using 2 phenotyping techniques: classic culture technique (CCT) and immunomagnetic separation (IMS). For the confirmation of the isolates at molecular levels, *invA* gene was detected in these isolates. The presence of *invA*, class 1 (Cls1) integrons, and integrase (*Int1*) genes was demonstrated by PCR assay; and the resistance of the isolated *Salmonella* spp. strains to antibiotics was determined by disk diffusion test. All the cultural and PCR results were evaluated together; *Salmonella* spp. were detected in a total of 64 (42.66%) chicken meat samples. Contamination rate was higher in carcasses (53.33%, $n = 75$) than in meat pieces (32%, $n = 75$). When results of standard culture were compared with IMS technique, IMS ($n = 54$) showed a clear superiority over the CCT ($n = 38$). A very high resistance rate (≥89.28%) to vancomycin, tetracycline, streptomycin, or nalidixic acid was found. Trimethoprim-sulfamethoxazole resistance was present in 32.14%. Relatively lower incidence of resistance (≤8.33%) to gentamicin, chloramphenicol, ampicillin, and ceftriaxone was observed. Concurrent resistance to at least 4 antibiotics was detected in 92.85% of the isolates. Cls1 integrons and *Int1* were positive in 80.95% and 95.23% of the isolates, respectively. However, *Int1* alone was detected in 15.47% ($n = 13$). In conclusion, the high prevalence of *Salmonella* spp. in chicken meat may pose a potential public health risk, and the presence of antibiotic-resistant *Salmonella* spp. isolate together with Cls1 integron and/or integrase might play an important role in horizontal antibiotic gene transfer.

Keywords: antibiotic resistance, chicken meat, integron, *Salmonella*

Practical Application: In this study, the presence of *Salmonella* spp. in 150 chicken meat samples, the presence of *invA*, antibiotic properties, and carriage of class I integrons and integrase genes were investigated. High prevalence of *Salmonella* spp. in consumed chicken meat can risk of public health from several ways due to (i) causing foodborne salmonellosis, (ii) multiple antibiotic resistance properties, (iii) potential transfer of drug resistance genes to other members of the Enterobacteriaceae and humans via Cls1 integron and/or integrase genes, and (iv) a very high resistance rate to nalidixic acid (initial steps of the development of ciprofloxacin resistance).

Introduction

Increasing poultry meat production and consumption is associated with certain public health problems. *Salmonella* spp. are also a serious safety concern for the food industry particularly for poultry and public health. According to CDC (2007; 2010) data, each year nearly one million people are affected through consumption of food contaminated with *Salmonella* spp. (CDC 2011). Similarly, EFSA (European Food Safety Authority) (2010) has reported that salmonellosis was the most common reported zoonotic disease in humans accounting for 131468 confirmed human cases. In the same report, *Salmonella* spp. were most often detected in fresh broiler (5.1%), turkey (5.6%), and pig meat (0.7%). Also, many studies in Turkey (Erol and others 2004; Efe and Gümüşsoy 2005; Yazıcıoğlu and others 2005; Hadimli and others 2006; Tanoğlu 2008) and in other countries (Capita and others 2003; Salehi and others 2005; Bada-Alambedji and others 2006; Minami and others 2010; Aslam and others 2012; Kim and others 2012; Ta and others 2012; Thai and others 2012) have showed a high prevalence (up to 88.4%) of contamination with *Salmonella* spp. in chicken meat samples.

In addition to the problem of food-borne salmonellosis, recent increase in global antibiotic resistance poses serious public health issues and increased economic burden due to increased morbidity and mortality in animals and humans. In the United States, the newly released 10th National Antimicrobial Resistance Monitoring System (NARMS) report describes alarming increases in antibiotic-resistant bacteria found on retail meats. The report has also confirmed that 80% of all antibiotics used in the United States are used not in humans but on food animals, most of which are perfectly healthy (NARMS 2011). Multidrug resistance (MDR) in bacteria has been associated with the use of antimicrobials for multiple purposes including treatment, prophylaxis (prevention), and growth promotion/increased feed efficiency in animals (McEwen and Fedorka-Cray 2002). Aside these purposes, an overwhelming majority of the antibiotics are used in primary animal production. The World Health Organization (2002) has also reported that about half of the antimicrobials produced globally is used in animal food, and according to data from Europe, approximately

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100 mg of antimicrobials are used in animals produced for per kg of meat for human consumption. Obviously, antimicrobial resistance in bacteria and the spread of antibiotic resistance via meat consumption have potentially serious implications from a global public health viewpoint (Ribot and others 2002).

Reservoirs of antibiotic resistance can interact between different ecological systems, and potential transfer of resistant bacteria or resistant genes from animals to humans may occur through the food chain (Witte 2000). In addition, mobile genetic elements in *Salmonella* strains, such as plasmids, transposons, and integrons play an important role in the evolution and dissemination of MDR (Boyd and others 2002).

Integrons are one of the genetic elements involved in the acquisition of antibiotic resistance. There are 2 main groups of integrons: Mobile integrons (MIs), related to antibiotic resistance cassettes, and Chromosomal Integrons (Cls), not related to antibiotic resistance with a few exceptions (Cambray and others 2010). Mobility of MIs is defined as being associated with mobile genetic elements (transposons or plasmids). Integrons are thus ideally suited for the dissemination and recombination of antimicrobial drug-resistance genes. Five different classes (Cls1 to Cls5) of MIs have been defined based on the sequence of the encoded integrases. Although only Cls1, 2, and 3 have been historically involved in the spread of multiresistance phenotypes, all 5 classes have been associated with antibiotic-resistance determinants. Cls1 integrons are the most widespread and clinically important, as they are detected in 22% to 59% of Gram-negative clinical isolates (Mazel 2006) and among genus of *Salmonella* (Michael and others 2006). The presence of integrase gene (*Int1)* is known to be an essential part of all integrons. A functional integrase is necessary to continue the catalytic process for the insertion/deletion of gene cassettes (Hanau-Bercot and others 2002). It is also known that the presence of integrase is potentially indicative of strains capable of recruiting antibiotic resistance genes (Di Conza and Gutkind 2010).

The *invA* gene is specific for the *Salmonella* spp. (Chen and Griffiths 2001), thus, it constitutes a suitable PCR target in diagnostic applications (Rhan and others 1992). In addition to this property, it is also a virulence gene. Virulence genes encode products that assist the organisms in expressing its virulence in the host cells. There are several virulent genes (*sef*, *pef, spv*, *mgtC, sop, stn, pip A, B, D,* and so on) have been among *Salmonella* species (Murugkar and others 2003) and of these, *invA* gene, a member of genetic locus (Salmonella Pathogenity Island 1-SPI1), allows *Salmonella* spp. to enter cultured epithelial cells. It is the first gene of an operon consisting of at least 2 additional invasion genes (Galán and others 1992). Lilic and others (2010) reported that the *Salmonella* protein InvA is one of the most highly conserved proteins of this core of critical the type III secretion system (T3SS) components. A number of Gram-negative bacterial pathogens utilize a highly specialized nanomachine termed as the T3SS to achieve a remarkable translocation of bacterial proteins across the cytoplasmic membranes and directly into the cytoplasm of the host organism (Cornelis 2006).

The aim of this study is to investigate (i) the presence of *Salmonella* spp. in chicken meat samples using 2 different methods: classic culture technique (CCT) and immunomagnetic separation (IMS) technique, and superiority over the other, (ii) to employ a confirmatory PCR assay, (iii) to determine antibiotic resistance profile of the isolates, and (iv) to detect Cls1 integron and integrase genes (*Int1)* of the isolates.

Materials and Methods

Sample collection

In the present study, a total of 150 chicken meat samples (75 carcasses and 75 pieces of meat) randomly collected from supermarkets in Samsun province between 2008 and 2009 were analyzed. The samples represented 12 different chicken meat producers in Turkey. All samples were transported under refrigerated conditions and were tested immediately for *Salmonella* spp.

Isolation procedure

For the 2 isolation methods, CCT and IMS, each whole chicken carcass was aseptically transferred to a sterile polyethylene bag and 225 mL Buffered peptone water (BPW-Oxoid, CM0509, Basingstoke, UK) was added to the samples for the pre-enrichment step. The carcasses were rinsed for 2 min (Baumgart 1986). Then, carcasses were removed aseptically and the remaining rinsate in the sampling bags was incubated at 37 °C for 24 h. Also, each piece of meat samples (25 g) were transferred to a sterile polyethylene bag containing 225 mL BPW, then homogenized (Interscience-bagmixer 400, Saint Nom, France) and incubated at 37 °C for 24 h. After the incubation, 0.1 mL of pre-enrichment broth was added to 10 mL Rappaport Vassiliadis (RV) enrichment broth (RV-Broth-Oxoid, CM0669, Basingstoke, UK) and incubated at 42.5 °C for 24 h for selective enrichment. Afterwards, they were streaked onto the media for CCT and IMS technique. The culture was streaked onto Brilliant green (Modified) agar (BGA-Oxoid CM 329, Suppl. SR 87, Basingstoke, UK) and incubated for up to 48 h at 37°C. After the incubation about 5 colonies suspected of being *Salmonella* spp. from each plate were selected and subcultured onto Tripticase Soy Agar (TSA-Oxoid-CM 131-L21, Basingstoke, UK). The IMS procedure was applied using immunomagnetic beads coated with anti-*Salmonella* antibody (Dynabeads anti-Salmonella; Dynal A.S., Norway) according to the manufacturer's instructions. Briefly, 20 μL of Dynabeads anti-*Salmonella* was added to a 1.5-mL eppendorf tube. Then, 1 mL of selective enrichment sample was added to it. The tube was loaded into the Dynal Magnetic Concentrator (MPC-S rack) which removed the magnetic plate. It was inverted 5 times to mix the sample and beads and incubated at room temperature for 10 min. The magnetic plate was inserted into the MPC^{TM}-S and 3 min were allowed for proper recovery of the beads. After that, the tube cap was opened, and the supernatant, as well as the remaining liquid in the tube, was aspirated and discarded carefully. The magnetic plate from the MPC-S was removed and 1 mL of wash buffer (PBS-Tween- Phosphate Buffered Saline in Tween 20, Sigma, P3563) was added into each eppendorf tube. The tube's cap was closed and the rack was inverted again several times to resuspend the beads. The same procedure was applied 2 more times. At the end of the IMS procedure, 100 mL of wash buffer was added to resuspend the beads-bacteria complex, and finally 50 μL of resuspended complex was spread onto BGA plates.

Identification procedure

Colonies were identified by Gram staining and standard biochemical tests (oxidase test, triple sugar iron agar-CM277; lysine iron agar-CM 381; urease test-urea Agar, CM 53; Simmons citrate-CM155, and ONPG-disc- DD13 ONPG). All the media that was used were provided by Oxoid Ltd., Basingstoke, United Kingdom. Presence of *Salmonella* spp. in the samples was

Table 1–Primers used in this study.

Amplified	Oligonucleotid sequence	Products (bp)	Reference
invA (genus specific)	F 5' – GTG AAA TTA TCG CCA CGT TCG GGC AA -3' R 5' – TCA TCG CAG CGT CAA AGG AAC -3'	284	Rahn and others (1992)
Class 1 Integron	5' GGC ATC CAA GCA GCA AG 3' 5' AAG CAG ACT TGA CCT GA 3'	Varied	Bass and others (1999)
Integrase *(IntI1)*	5' CCT CCC GCA CGA TGA TC 3' 5' TCC ACG CAC TGT CAG GC 3'	280	Bass and others (1999)

tested using previously reported methods (Andrews and Hammack 2011). Confirmation was based on agglutination with *Salmonella* spp. antisera polyvalent antiserum (Difco 2264-47-2), and subsequent reconfirmation was done using Microbact-Gram-Negative Bacillus Identification System (Oxoid, GNB 24 E).

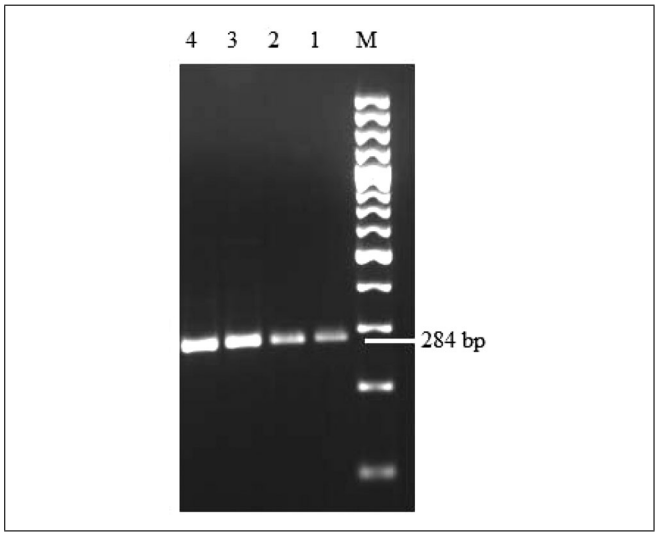

Figure 1–The determination of the *invA* gene in the *Salmonella* strains isolated from chicken meat samples using PCR technique. M: Marker; 1, 2, 3, and 4 *invA* gene positive isolate.

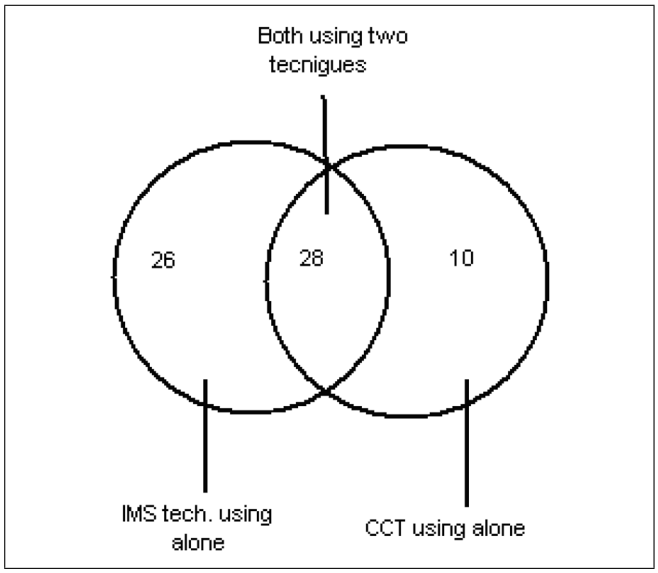

Figure 2–Number of *Salmonella* spp. positive samples detected by 2 techniques. A total 64 *Salmonella* spp. positive samples; 54 samples detected in using IMS and confirmed using PCR assay, 38 samples detected in using CCT and confirmed using PCR assay.

All the isolates were stored at –80 °C in cryovials containing Brain heart infusion broth containing 25% glycerol (BHI-Oxoid CM0225).

Single Target PCR Assay for the Verification of *Salmonella* spp.

PCR analysis. DNA templates used for PCR were prepared by boiling bacterial cultures. For the detection of genus-specific *invA* gene (Rham and others 1992) of *Salmonella* spp., a single target PCR assay described by Salehi and others (2005) was used. S. Typhimurium (ATCC 14028) was used as a positive control. During the procedures, a gradient thermocycler (Bio Rad-MJ Mini-PTC-1148, Singapore) was used.

Detection of class 1 integron (Cls1) and integrase (IntI1) genes

The single target assays for each gene were applied according to the method described by Bass and others (1999).

The nucleotide sequences (invA, Cls1, and class 1integrase) of the primers and the predicted product sizes are depicted in Table 1.

Antimicrobial susceptibility testing

The susceptibilities of the isolates to gentamicin (10 μg), vancomycin (30 μg), chloramphenicol (30 μg), trimethoprim-sulfamethoxazole (25 μg), streptomycin (10 μg), ceftriaxone (30 μg), tetracycline (30 μg), nalidixic acid (30 μg), or ampicillin (10 μg) were determined using the Kirby-Bauer disc diffusion method. The resistance levels were evaluated the definitions proposed by Clinical and Laboratory Standards Institute (CLSI 2007, 2010) as follows: susceptible (S), intermediate-resistant (I), or resistant (R).

Chi-square test was employed to compare the resistant and susceptibility isolates against the antibiotics. $P < 0.001$ was considered as statistically significant.

Results

All the cultural and PCR results (Figure 1) were evaluated together, and 86 *Salmonella* spp. were detected in a total of 64 (42.66%) chicken meat samples (in 40 [53.33%] of the carcass and in 24 [32%] of the meat piece samples). The study results are shown in Figure 2. When results of standard culture technique were compared with the IMS technique, IMS technique could detect *Salmonella* spp. in 54 (36.00%) samples (32 carcasses and 22 meat pieces) and the CCT method could detect *Salmonella* spp. in 38 (25.33%) samples (23 carcasses, 15 meat pieces). Although *Salmonella* spp. were detected in 3 chicken meat samples using IMS technique, *invA* gene was not determined.

The antibiotic susceptibility testing showed higher resistance rates to vancomycin (98.8%), tetracycline (91.66%), streptomycin (89.28%), and nalidixic acid (89.28%). Resistance to trimethoprim-sulfamethoxazole was present in 32.14% of the isolates. In contrast, lower rates of resistance were observed for

Table 2–Antibiotics resistance profiles of the *Salmonella* isolates.

Antibiotics	R (n) (%)	I (n) (%)	S (n) (%)
Gentamicin (10 μg)	4/84 (4.76)	–	80 (95.24)
Vancomycin (30 μg)	83/84 (98.8)	–	1 (1.19)
Chloramphenicol (30 μg)	6/84 (7.14)	–	78 (92.86)
Trimethoprim-sulfamethoxazole (25 μg)	27/84 (32.14)	–	57 (67.86)
Streptomycin (10 μg)	75 (89.28)	6 (7.14)	3 (10.72)
Ceftriaxone (30 μg)	1/84 (1.19)	–	83 (98.81)
Tetracycline (30 μg)	77/84 (91.66)	–	7 (8.34)
Nalidixic acid (30 μg)	75/84 (89.28)	–	9 (10.72)
Ampicillin (10 μg)	7/84 (8.33)	–	77 (91.67)

gentamicin (4.76%), chloramphenicol (7.14%), ampicillin (8.33%), and ceftriaxone (1.19%). Statistically significant differences were determined between the antibiotics and the resistance of *Salmonella* strains to the analyzed antibiotics ($P < 0.001$) (Table 2).

Multiple resistances (≥4 antibiotics) were found in 78 out of 84 (92.85%) isolates. One isolate was resistant to all antibiotics tested, except for trimethoprim-sulfamethoxazole; 2 isolates (2.38%) were resistant to 7 antibiotics, 6 isolates (7.14%) to 6 antibiotics, 21 (25%) isolates to 5 antibiotics (vancomycin, trimethoprim-sulfamethoxazole, streptomycin, tetracycline, nalidixic acid), 45 (53.7%) isolates to 4 antibiotics (vancomycin, streptomycin, tetracycline, and nalidixic acid), 1 isolate to 3 antibiotics, 2 isolates to 2 antibiotics, and the other 3 isolates to a single antibiotic (Table 3).

Cls1 integrons were present in 80.95% (68/84) of the isolates with product patterns between 400 bp and 1200 bp, most being 900 bp or 1100 bp (Figure 3). The isolates also demonstrated more than one Cls1. In total, 80 (95.23%) isolates carried *Int1* (Figure 3). This gene also existed in 67 (79.76%) of 68 isolates containing Cls1. However, integrase gene alone was present in 13 (15.47%) isolates. Thus, most of the isolates ($n = 81$) carried Cls1 integron or *Int1*. Although 4 isolates did not carry any Cls1 integron or *Int1*, they were resistant to 4 antibiotics (vancomycin, streptomycin, tetracycline, and nalidixic acid).

Discussion

Global incidence of *Salmonella* spp. infections in humans has shown a drastic increase in recent years. According to a *Salmonella* Atlas in the United States, it is estimated that 1.2 million salmonellosis occurs each year, with more than 23000 hospitalizations and 450 deaths (CDC 2013). Therefore, it is not surprising to observe the plenitude of studies in other countries, as well as in Turkey, that aim to examine the prevalence of *Salmonella* spp. contamination in chicken meat. Some of the results from these studies are summarized in Table 4 and 5. As can be seen from these results, *Salmonella* spp. were included in 4.2% to 88.4% of the cases. Similar to our findings, the generally high isolation rate of *Salmonella* spp. in chicken meat samples indicates the fact that chicken meat has been one of the most important sources of human salmonellosis.

The wide variation in these study findings mentioned above may be due to a number of factors including geographical location, methodological differences, seasonal variations, as well as farming and slaughtering conditions. In the present study, IMS was able to detect *Salmonella* spp. in 54 (36.00%) samples and the CCT method was able to detect *Salmonella* spp. in 38 (25.33%) samples. When the results of the standard culture were compared with the results from the IMS technique, the IMS showed a clear superiority over the CCT (Figure 3). The reason may depend on eliminating competitive flora. A similar advantage for this approach has previously been emphasized by other authors including Cudjoe and Krona (1997). Success of the isolation is also determined by the selective enrichment broth used. For example, the IMS-RV broth combination has been reported to provide a higher isolation rate than direct IMS methods without selective enrichment (Ribeiro and others 2002). Success of isolation is also dependent on certain other factors such as the sampling site (skin or meat piece), sampling either from the whole carcass or meat pieces, or the swap technique used. In a study by Capita and others (2003), an isolation rate of 49% has been detected for *Salmonella* spp. in chicken meat, compared to 55% in chicken skin. In our study, a higher isolation rate was found in whole chicken carcasses ($n = 41$) than in meat piece samples.

Antimicrobial resistance represents a serious public health problem. Food may act as a vector for the transfer of antimicrobial resistant bacteria and antimicrobial resistance genes to humans (Verraes and others 2013). The present study, as well as other study results reported from around the world, also supports this view. For instance, from Turkey, Yazıcıoğlu and others (2005) have found that among 58 *Salmonella* spp. isolates of avian origin, the most common resistance rates were observed against nalidixic acid and streptomycin. Kılınç and Aydın (2006) have reported that none of the 61 *Salmonella* spp. isolates were resistant to trimethoprim-sulfamethoxazole, tetracycline and gentamicin, while 12 (19.67%) out of 61 isolates were resistant to ampicillin. Although the results of the present study are similar to that of Yazıcıoğlu and others (2005), they were in contrast with those of Kılınç and Aydın (2006). In our study, following vancomycin, the highest resistance rates were observed against tetracycline (91.66%), streptomycin (89.28%), and nalidixic acid (89.28%). One possible reason for the high incidence of tetracycline and streptomycin resistance may be the use of subtherapeutic doses of these antimicrobials (particularly tetracycline) in feeds.

Ceftriaxone is a member of cephalosporin family of antibiotics which are especially important from several aspects (Yan and others 2002). In the present study, only a single *Salmonella* spp. isolate showed resistance to ceftriaxone.

Nalidixic acid plays a role in the initial steps of the development of ciprofloxacin resistance and a very high rate of nalidixic acid resistance was observed in the isolates in our study (89.28%), where resistance to ciprofloxacin was not tested.

According to EFSA (2013), in 2011, a total of 8 European Member Countries (MSs) have reported that the resistance levels of broiler meat origin *Salmonella* spp. to ampicillin, sulfonamides, and tetracyclines were high at 20.6%, 44.8%, and 43.7%, respectively. The results of the study showed that the resistance rates were 8.33% (ampicillin), 32.14% (trimethoprim-sulfamethoxazole), and 91.66% (tetracyclines). In contrast to ciprofloxacin resistance rates (50.1% and 1.19% ceftriaxone resistance rates for MSs Countries and the present study results, respectively), resistance rates to nalidixic acid in the present study were relatively higher (89.28%) than the rates (48.8%) found in 8 MSs countries. Similar results from both this and the studies of MSs Countries were obtained for chloramphenicol and gentamicin.

Table 3–Distribution of antibiotic-resistance rates among *Salmonella* spp. isolates.

Nr of isolates	Ratio (%)	Nr of resistance antibiotics	Resistance antibiotics names
1	1.19	8	Gentamicin, vancomycin, chloramphenicol, streptomycin, ceftriaxone, tetracycline, nalidixic acid, ampicillin
2	2.38	7	Gentamicin, vancomycin, chloramphenicol, trimethoprim-sulfamethoxazole, streptomycin, tetracycline, nalidixic acid
3	3.57	6	Vancomycin, trimethoprim-sulfamethoxazole, treptomycin, tetracycline, nalidixic acid, ampicillin
1	1.19	6	Vancomycin, trimethoprim-sulfamethoxazole, treptomycin, tetracycline, nalidixic acid, ampicillin
2	2.38	6	Vancomycin, chloramphenicol, trimethoprim-sulfamethoxazole, streptomycin, tetracycline, nalidixic acid
21	25.00	5	Vancomycin, trimethoprim-sulfamethoxazole, streptomycin, tetracycline, nalidixic acid
45	53.57	4	Vancomycin, streptomycin, tetracycline, nalidixic acid
2	2.38	4	Vancomycin, chloramphenicol, streptomycin, ampicillin
1	1.19	4	Gentamicin, vancomycin, chloramphenicol, tetracycline
1	1.19	3	Vancomycin, trimethoprim-sulfamethoxazole, streptomycin
2	2.38	2	Vancomycin, streptomycin
3	3.57	1	Vancomycin

Table 4–*Salmonella* spp. prevalence and dominate serotypes in chicken meats, Turkey.

Provinces of Turkey	Sources (*n*)	Prevalence	Dominate serotypes	References
Ankara	Chicken skin, leg and brisket meats) ($n = 200$)	%16	–	Tanoğlu (2008)
Konya	Chicken meats ($n = 168$)	%32.73	–	Hadimli and others (2006)
Ankara	Chicken neck and meat ($n = 662$)	%8.7	*S.* Enteritidis *S.* Virchow *S.* Typhimurium	Yazıcıoğlu and others (2005)
Ankara	Boriler skin, leg and brisket meats ($n = 50$)	%26 %18 %16		Efe and Gümüşsoy (2005)
Ankara	Chicken carcass ($n = 69$)	%88.4		Erol and others (2004)

MDR to the traditional first-line antibiotics such as ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole has been defined for *Salmonella enterica* (Crump and Minz 2010). Although resistance to 2 of these 3 antibiotics was found in the same isolates in our study, no triple resistance against these 3 antibiotics in the same isolate was observed. According to CDC (2007) report, the most common *S. enterica* multidrug-resistance pattern in 2004 was ACSSuT (resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline). In this report, multiple drug resistance (≥4 antibiotics) has found in all 78 isolates. In our study, 30 of the isolates were resistant to at least 5 antibiotics and other 48 isolates were resistant to 4 antibiotics (Table 3).

Molecular analysis of the isolates was used to determine the presence of some characteristics that are associated with the

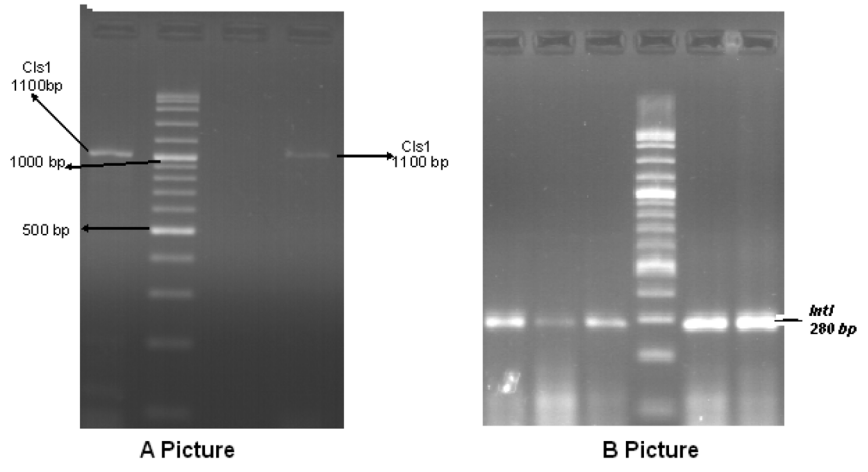

Figure 3–The determination of the integron (Cls1) (A Picture) and integrase gene (*IntI*) (B Picture) in the *Salmonella* strains isolated from chicken meat samples. A Picture—Lane 1: 1100 bp Cls1 positive samples, Lane M: Marker (100 bp: between 100 and 3000 bp), Lane 2: Cls1 negative samples, Lane 3: 1100 bp Cls1 positive samples; B Picture—Lanes 4, 5, and 6: integrase positive samples, Lane M: Marker (100 bp), Lanes 7 and 8: integrase positive samples.

Table 5–*Salmonella* spp prevalence and dominate serotypes in chicken meats, World.

Country	Part of chicken meat	Prevalence	Dominate serotypes	References
Vietnam	Chicken carcass ($n = 1000$)	45.9%	–	Ta and others (2012)
North Vietnam	Chicken meat ($n = 268$)	42.9%	*S.* Anatum *S.* Infantis *S.* Derby	Thai and others (2012)
South Korea	Chicken meat ($n = 210$)	57.4%	*S.* Enteritidis *S.* Montevideo	Kim and others (2012)
Canada	Chicken meat ($n = 206$)	40%	*S.* Hadar	Aslam and others (2012)
Thailand	Chicken carcass ($n = 109$)	57%		Minami and others (2010)
Senegal	Chicken carcasses ($n = 120$)	62.5%	*Salmonella* spp. *S.* Kentuchy	Bada-Alambedji and others (2006)
Persia	Chicken carcass ($n = 192$)	15.6%		Salehi and others (2005)
Spain	Chicken carcass and pieces meat	49%		Capita and others (2003)
America	Chicken carcass ($n = 212$)	4.2%	–	Zhao and others (2001)

presence of antimicrobial resistance. It has been reported that MDR among *Salmonella* serotypes and other clinical isolates is often associated with the presence of Cls1 integrons (Leverstein-van and others 2002; Wannaprasat and others 2011). The presence of genes within integrons coding for antibiotic resistance and the association of antibiotic resistance phenotypes with the presence of integrons has been documented by several authors (Miko and others 2005). The present study results also showed that 80.95% and 95.23% of the *Salmonella* spp. isolates were found to carry Cls1 integron and *Int1*, respectively. So, we can say that these MDR *Salmonella* spp. isolates can be transferable to humans via chicken meat or can spread to environment. Similar results have been reported from Germany, Spain, UK, Japan, China, Ethiopia, and Iran where the prevalence of Cls1 integrons among the MDR *Salmonella* serovars isolated from various types of sources such as food of animal origin and human materials were found 11% and 65% (Guerra and others 2000; Randall and others 2004; Zhang and others 2004; Ahmed and others 2005; Miko and others 2005; Molla and others 2007; Firoozeh and others 2011). Another aspect is that the presence of resistant *Salmonella* spp. isolates in conjunction with the detection of Cls1 integron and/or *Int1* in this study suggests that antimicrobial resistance may be attributed to a relatively high number of resistant strains circulating in the chicken farms.

In the present study, we checked for the presence of *invA* gene to confirm the *Salmonella* spp. genetically (Chen and Grifiths 2001). In addition to concerns previously expressed, *invA* gene also plays an important role in the invasion of *Salmonella* to the host tissue (Khan and others 2000). In this study, a cocktail consisting of the 5 *Salmonella* spp. isolates obtained from the present study was prepared to test for their effects on human colon adenocarcinoma cells (HT-29) *in vitro*. We found that these isolates had the ability to adhere to and invade into human colon adenocarcinoma cells (HT-29) (unpublished data).

Conclusion

Salmonellosis and the antimicrobial resistant in the bacteria in veterinary and human medicine constitute a big global problem that includes Turkey. The data presented in this study demonstrated that retail chicken meat samples were commonly contaminated with *Salmonella* spp. Therefore, chicken meat may be a potential vehicle of transmission of the bacteria to humans. In addition, data from the present study also showed that most of the *Salmonella* spp. isolates were resistant to multiple antimicrobial drugs. Class 1 integron and integrase genes were also present in a total 80.95% and 95.23% *Salmonella* spp. isolates. The role of integrons as mobile genetic elements playing a central role in horizontal transfer of antibiotic resistance has been well documented. Also, Cls1 integron (transferable plasmids) are considered to be the main mechanism for the rapid spread of multidrug-resistant phenotypes among *Salmonella* spp. and other Gram-negative bacteria (Labbate and others 2009). So, the high prevalence of integron in drug- or multidrug-resistant *Salmonella* spp. in our study indicated that these mobile genetic elements were common among *Salmonella* spp. and drug resistance could be associated with the presence of class 1 integrons in *Salmonella* spp. As a consequence, transfer of antimicrobial resistance genes between *Salmonella* isolates after ingestion by humans may occur.

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Author Contributions

Belgin Siriken contributed substantially for the study designing, all of the laboratory study stage, data analysis and interpretation of the results, and manuscript writing and design. Haldun Türk collected samples and all of the laboratory study stage except molecular study, data analysis, and interpretation of the results data with Siriken. Tuba Yıldırım supported language correction and antimicrobial evaluation of the isolates. Belma Durupınar supported evaluation of antimicrobial resistant data of the isolates and writing levels of this section and tables. İrfan Erol supported evaluation data and manuscript design.

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