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SOME SPECIFIC MICROBIOLOGICAL PARAMETERS AND PREVALENCE OF *SALMONELLA* spp. IN RETAIL CHICKEN MEAT FROM ERZURUM PROVINCE, TURKEY AND CHARACTERIZATION OF ANTIBIOTIC RESISTANCE OF ISOLATES

ALGUNS PARÂMETROS MICROBIOLÓGICOS ESPECÍFICOS E PREVALÊNCIA DE SalmonellA spp. *CORTES DE CARNE DE FRANGO DA PROVÍNCIA DE ERZURUM, TURQUIA E CARACTERIZAÇÃO DA RESISTÊNCIA AOS ANTIBIÓTICOS DE ISOLADOS*

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ABSTRACT: Specific microbiological parameters and the presence of *Salmonella* spp. were investigated in 72 chicken meat samples (36 wings and 36 drumsticks) collected from markets and butcher shops. The specific microbiological parameters were determined using a conventional cultural method and the presence of *Salmonella* spp. in chicken samples was determined using conventional and immunomagnetic separation (IMS)-polymerase chain reaction (PCR) methods. In addition, antimicrobial susceptibility of the isolates was revealed using the Kirby-Bauer disc diffusion method. The results indicated that 30 of the 72 samples were positive for *Salmonella* spp. by the conventional method, and 42 of the 72 were positive by the IMS-PCR method. However, 30 of the 72 samples were positive for *Salmonella* spp. by both methods. The *Salmonella* spp. isolates were confirmed by the VITEK2 Compact System and PCR. The susceptibilities of the isolates against 10 antibiotics were determined. The results indicated that isolates (27/30) showed the highest susceptibility to gentamycin (90.00%), while the highest resistance was to nalidixic acid and tetracycline at the 100 and 93.34% levels, respectively. These results indicate a high prevalence of *Salmonella* spp. in poultry meat from Erzurum city, Turkey, and the antimicrobial resistance profile of these isolates should be considered for public health. The results also show that the IMS-PCR technique was superior to the conventional method for detecting *Salmonella* in poultry meat.

KEYWORDS: Chicken meat. *Salmonella*. IMS. PCR. Antimicrobial.

INTRODUCTION

Chicken is one of the most popular food products worldwide, because of nutritional, sensorial and economic factors. Chicken is widely consumed in homes and fast-food establishments, but can become contaminated during processing. The contamination of poultry products with *Salmonella* and other microorganisms is due to unhygienic conditions during the production, processing, distribution, marketing and preparation stages (DOOKERAN et al., 2012).

The genus *Salmonella* includes short rod-shaped, facultative anaerobe, Gram-negative bacteria. Warm-blooded animals and humans are natural hosts for *Salmonella* spp. Detecting *Salmonella* spp. during production and before consumption is important to prevent food-borne salmonellosis. A *Salmonella* infection in humans is usually caused by consuming undercooked meat or other cross-contaminated foods, such as vegetables, milk and eggs (HASSANEIN et al., 2011). According to a report published by the Centres for Disease Control and Prevention (CDC), it is estimated that about 1.2 million people in the US have been exposed to *Salmonella* infections, and that an average of 23.000 hospitalisations and 450 deaths occur from these infections. The prevalence rates of *Salmonella* spp. in chicken meat sold in Turkey are 34-68.75%. Not only in Turkey, but in most developing countries, the absence of an epidemiological surveillance system for salmonellosis cases makes it difficult to effectively assess prevalence (KÄFERSTEIN, 2003). However,

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1.993 cases of gastroenteritis in Turkey were due to *Salmonella* spp. in 2008, and the number increased to 2.307 in 2011, according to unpublished data from the Department of Communicable Diseases of the Turkish Public Health Institution (THSK, 2015).

Salmonellosis is one of the most important zoonotic bacterial food-borne infections worldwide. *Salmonella* causes severe illness in infants, elderly humans and immunocompromised patients. Generally, *Salmonella* infections are related to infected animals' faeces or food products of animal origin. Chicken and other poultry meat and eggs are the most significant sources of *Salmonella* compared with other food products. *Salmonella* causes gastrointestinal illness, substantial morbidity and economic burden worldwide. The clinical symptoms of salmonellosis are characterised by fever, diarrhoea, nausea, vomiting, abdominal pain, headache and occasional constipation 12-72 h after consuming contaminated food (VOSE et al., 2011).

Increased public awareness about food-borne illness has necessitated the development of rapid, sensitive and specific techniques for detecting these food-borne pathogens. Isolation and identification of *Salmonella* from food samples requires approximately 7 days using traditional cultural techniques. In recent years, more rapid and sensitive methods have been developed to detect and identify *Salmonella* in chicken meat, including immunoassays, electrical techniques and nucleic acid analyses (BENNETT et al., 1998). Among these, immunomagnetic separation (IMS) and polymerase chain reaction (PCR) have been accepted as a potential approach for detecting these pathogens (YANG et al., 2010).

IMS has been successfully used to separate and concentrate target microorganisms from food samples using magnetic beads coated with specific antibodies to a target microorganism. This method is a quite rapid, specific and technically simple approach, as the target organism is captured by the magnetic particles and removed from the media by a applying a magnetic field (LYNCH et al., 2004). Finally, the microorganism is removed from the food debris and other competing microorganisms and is subjected to an enzyme linked immunosorbent assay or PCR analysis (HAGREN et al., 2008 ; LYNCH et al., 2004).

PCR detects pathogens from foods within a few hours and is a rapid alternative method to detect *Salmonella* (MOGANEDI et al., 2007). Although PCR is a sensitive, rapid technique, it can be inhibited by several factors, including food components, urine and bile salts; thus, different approaches, such as IMS, have been used to remove these substances prior to PCR (SCHEU et al., 1998). IMS isolates *Salmonella* from other microbes and facilitates removal of PCR inhibitors of different sizes. The combination of different rapid methods for separating and concentrating specific bacteria facilitates direct detection of pathogens in foods. The combination of IMS and PCR (IMS-PCR) is considered quite accurate and rapid for isolating pathogens (TABAN; AYTAC, 2009).

The *invA* gene region is located in the *Salmonella* pathogenicity 1B island and is necessary for *Salmonella* to invade epithelial cells (Lei et al., 2015). This genomic region, which is found in almost all *Salmonella* serovars, is a powerful target for detecting *Salmonella* (JEONG et al., 2011).

Antimicrobial resistance has increased among food-borne pathogenic microorganisms during recent decades (TEUBER, 2001). This increase is caused by irregular use of antimicrobials in food-producing animals and the random use of antibiotics by humans (BYWATER, 2004). Antibiotic-resistant *Salmonella*-related data are needed to assess the potential effect of resistant isolates (three or more antibiotic-resistant strains) isolated from raw chicken meat on public health (NAIR et al., 2018).

The aim of this study was to determine the general microbiological quality characteristics and presence of *Salmonella* spp. in different chicken meat samples obtained from supermarkets and butcher shops. In addition, this study compared and evaluated a conventional method with IMS-PCR analysis for detecting *Salmonella* spp. in chicken meat samples, and determined the antibiotic susceptibility and resistance of *Salmonella* spp. isolated from samples against 10 different antimicrobial agents.

MATERIAL AND METHODS

Material

In total, 72 packed chicken meat samples were collected from 19 different local markets and retail stores, in Erzurum city, Turkey during the May-December 2016. All samples were transferred to the laboratory under cold chain within one hour and microbiological analyses were carried out on the same day.

Microbiological Analysis

Microbiological analysis were performed according to methods of Turkish Standard Institute number of 1069 standard (TSE, 2016). Ten g of chicken meat was weighed for each sample and transferred to sterile stomacher bag which contained

90 ml of sterile Ringers (MERCK, 115525) ¼ solution and mixture was homogenized by a stomacher blender (IUL Instruments, Barcelona, Spain) for 90 s. Then a series of 10-fold dilutions was prepared in tubes and each of the diluted sample (0.1 mL) was plated on proper growth media except for total coliforms (coliforms were sown by pouring method). Total aerobic mesophilic bacteria were enumerated on Plate Count Agar (PCA) (Merck, 105463), at 35±1°C for 48-72 h ; total coliforms were counted on Violet Red Bile (VRB) Agar (Oxoid, CM0107B) at 35±1°C for 18-24 h ; *Enterococcus* was determined on Kanamycin Aesculin Azide (KAA) Agar (Oxoid, CM0591) at 35±1°C for 24 h ; for *Staphylococcus aureus* enumeration, Baird Parker Agar (BPA) (Merck, 1.05406) that supplemented with egg yolk telluride was used and incubated at 37±1°C for 24 to 48 h . *Micrococcus/Staphylococcus* was counted on Mannitol Salt Agar (MSA) (Oxoid, CM0085) at 30±1°C for 24 h; *Pseudomonas* was determined on Pseudomonas Selective Agar (Merck, 1.07620) supplemented with CFC (Merck, 1.07627) at 25°C for 48 h; yeast and moulds were counted on Rose Bengal Chloramphenicol (RBC) Agar (Merck, 1.00467) at 25°C for 5-7 d.

Bacterial Strain

Positive control used in PCR assay was obtained from Turkey Public Health Institution Microbiology Reference Laboratories (*Salmonella typhimurium* RSSK 95091).

Isolation and Identification of *Salmonella* spp.

Conventional Method

Isolation and identification of *Salmonella* spp. was performed according to method of ISO (2002). Briefly, 25 g of meat sample was transferred to filtered stomacher bags containing 225 mL of sterile buffered peptone water (Merck, 107228) and homogenized with masticator (Neutec Masticator, Neutec Group, Inc., Farmingdale, NY) for 90 s. Homogenized samples were incubated for pre-enrichment during 24 h. Then, 1 mL was transferred to tube containing Muller-Kauffmann tetrathionate/novabiocin broth (MKTTn) (Oxoid, CM1048) supplemented with novobiocin (Oxoid, SR0181) in 10 mL volume and 0.1 mL of pre-enriched solution was transferred to tube containing Rappaport-Vassiliadis (RV) medium (Merck, 1.07700) in 10 mL volume. For selective enrichment, the tubes with RV were incubated at 41.5°C for 24 h and inoculated tubes with MKTTn were incubated for 37°C for 24 h. Following the incubation, the enriched samples were streaked onto Xylose Lysine Tergitol-4 (XLT-4) Agar (Oxoid, CM1061) supplemented with tergitol (Oxoid, SR0237) and Xylose Lysine Deoxycholate (XLD) Agar (Merck 1.05287) and incubated at 37°C overnight. The colonies with a black centre with pinky-reddish periphery on XLD agar and black or black-centred with a yellow periphery on XLT4 Agar were accepted as suspicious for *Salmonella*. The suspicious colonies of *Salmonella* spp. were selected and identified by Triple Sugar Iron Agar (TSIA) (Oxoid, CM0277B), Lysine Iron Agar (LIA) (Oxoid, CM0381) and urea broth (Merck, 1.08483). Following the incubation at 37ºC for 24 h, typical reaction on TSIA (alkaline slant, acid butt, positive H_2S and positive/negative gas) and LIA (alkaline slant, alkaline butt, positive H_2S) and urea negative cultures were evaluated as suspicious for *Salmonella.* The other biochemical tests were performed by using GN Cards (BioMérieux, Inc., Craponne, France) including 64 different test substrates on VITEK 2 Compact system (BioMérieux) for verifying the isolates. For this purpose, the isolates were incubated at 37°C for 24 hours on blood agar. A sufficient number of colonies from pure culture were suspended in a polystyrene tube containing 3.0 mL of sterile saline solution (0.45%, pH 4.5). The McFarland turbidity of solution was adjusted to 0.5 using a turbidity meter. Then, the suspension was loaded on the GN cards. Identification of presumptive *Salmonella* spp. isolates were performed on VITEK2 Compact System (BioMérieux, Marcy l'Étoile, France) within 3 h using fluorescence reading of GN cards. VITEK2 Compact System Software identified the isolates as *Salmonella* spp. at the level of 97-99% probability.

IMS Method

Salmonella spp. were separated from pre-enriched samples by Dynabeads® anti-*Salmonella* (ThermoFischer Scientific, 71002) according to the manufacturer's instruction. Briefly, 20 µL of Dynabeads® anti-*Salmonella* was transferred into 1.5 mL of sterile eppendorfs. After, 1 mL pre-enriched samples were added to eppendorfs and incubated with gentle agitation for 10 min. Then, a magnetic plate was placed on the MPC-S rack and recovery of the beads was performed for 3 minutes. The IMS beads-bacteria complex on the tube wall were washed with 1 mL wash buffer (PBS with 0.05% Tween-20) following the removal of the magnetic plate from the MPC-S rack. The washing process was repeated twice.

Post-IMS

DNA extraction from the Dyne bead-bacterial complex was performed by the boiling method. For this purpose, 100 µL of Tris-EDTA buffer solution (pH, 8.0) containing dyne bead-bacteria was boiled for 10 min. At the end of the boiling, the samples were cooled on ice and centrifuged at 10.000 x*g* for 15 sec. The supernatant was used template in PCR.

PCR Method

PCR primers (*invA*FW: 5'-ACA GTG CTC GTT TAC GAC CTG AAT-3'; *invA*RV 5'-AGA CGA CTG GTA CTG ATC GAT AAT-3') were used to specifically amplify a 284-bp genomic fragment of the *invA* gen, which is highly specific for *Salmonella* spp. for PCR assays of the isolates. The PCR amplifications were performed in a total volume of 15 µL solution containing 2 µL of template DNA, 1×PCR buffer (Sigma), 0.25 mM $MgCl_2$ (Sigma), 200 µM (each) dNTP (Sigma), 10 pmol of each primer, 1.25 U of Taq polymerase (Sigma). The PCR cycle condition was an initial denaturation at 95°C for 10 min; 30 cycles of 95°C 30 s, 55°C 30 s and 72°C 30 s; and a final extension at 72°C for 5 min. The amplified products were detected by electrophoresis in a 1% agarose gel in Tris/Borate/EDTA Buffer (TBE, pH 8.3) pre-stained with ethidium bromide under UV light using Gel Doc™ XR+ Gel Documentation System (BioRad, USA).

Antibiotic Susceptibility Test of Obtained Isolates

The antimicrobial susceptibilities of the obtained isolates were determined by agar disk diffusion method proposed by Clinical & Laboratory Standards Institute (CLSI, 2012). Then, isolates were spread onto petri dishes containing Nutrient agar and incubated for 18 hours at 35±1ºC. The colonies were diluted in 0.85% physiological saline and the turbidity of the inoculum was adjusted to 0.5 McFarland standard. The suspension was then streaked onto surface of Müeller Hinton Agar in petri dish with a sterile swap. The following antibiotic discs: ampicillin (AMP, Oxoid CT003B), chloramphenicol (C, Oxoid CT013B), ciprofloxacin (CIP, Oxoid CT425B), gentamicin (CN, Oxoid CT024B), kanamycin (K, Oxoid CT026B), nalidixic acid (NA, Oxoid CT031B), streptomycin (S, Oxoid CT047B), sulfamethox/trimethoprim (SXT, Oxoid CT052B), tetracycline (TE, Oxoid CT054B) trimethoprim (W, Oxoid CT076B) were dispensed onto the inoculated surface within 15 minutes. Then the petri dishes were incubated in ambient air at 35°C for 18 h. The nearest millimeter was measured with a ruler following the incubation. The results were interpreted according to the standards of Clinical & Laboratory Standards Institute (CLSI Guidelines). Antibiotic susceptibility of isolates was evaluated as resistance, intermediate and sensitive (CLSI, 2012).

Statistical Analysis

Statistical analysis was performed using SPSS Software Programme (SPSS software, version 20). P value of 0.05 or less was considered to indicate a statistically significant difference.

RESULTS

The general microbial counts (log cfu/g) in the chicken meat samples collected from 19 retail markets are presented in Table 1.

Table 1. The general microbiological properties and presence of *Salmonella* spp. in chicken meat samples (log cfu/g).

Sample ID	TAMB	Pseudomonads	Mold and yeast	Coliform	Enterococci	Staph-Micrococci	IMS-PCR Method	Conventional Method
1	7.28	6.09	5.90	4.55	2.28	4.40	+	+
2	7.28	5.08	4.58	4.15	<2	4.30	+	-
3	4.48	3.00	2.23	2.00	<2	<2	+	+
4	7.35	5.06	4.90	6.00	4.11	4.00	+	-
5	5.00	4.32	0.82	<2	<2	4.00	+	+
6	6.39	6.28	5.00	4.92	2.30	4.00	-	-
7	6.36	4.21	1.27	<2	2.48	3.11	+	+
8	5.85	4.32	4.00	4.97	2.60	2.85	-	-
9	7.30	3.90	3.30	5.32	3.48	4.85	+	+
10	1.27	<2	<2	3.30	<2	<2	+	+
11	7.00	<2	4.31	<2	<2	<2	-	-
12	6.21	<2	<2	4.30	3.00	<2	-	-
13	4.00	<2	<2	3.48	2.30	3.35	-	-
14	5.19	4.11	4.00	5.13	2.78	3.18	-	-

15	6.21	6.26	5.65	<2	<2	<2	+	-
16	6.23	2.22	5.74	4.09	<2	5.65	-	-
17	6.82	2.33	5.60	4.37	1.30	4.66	-	-
18	6.35	5.74	4.90	4.34	1.78	<2	-	-
19	7.10	6.40	4.70	4.65	1.48	<2	-	-
20	6.72	5.67	5.00	5.60	<2	<2	+	-
21	4.63	4.04	3.48	2.57	<2	3.00	+	-
22	6.03	3.56	3.78	3.06	1.78	<2	+	-
23	6.83	4.28	3.90	6.33	3.71	4.29	+	-
24	6.01	2.00	3.23	4.27	3.28	3.76	+	-
25	9.41	4.78	5.16	7.61	<2	4.60	+	+
26	10.05	4.30	5.48	6.52	3.70	4.30	+	+
27	4.00	<2	<2	<2	<2	2.00	-	-
28	4.00	2.00	3.00	3.70	3.18	2.48	+	+
29	5.00	2.30	<2	3.20	2.00	2.00	+	-
30	7.95	3.65	4.20	4.32	2.70	2.30	+	+
31	6.20	5.52	3.00	5.20	2.85	<2	+	+
32	6.28	5.30	4.00	4.62	2.78	<2	+	-
33	6.00	4.15	5.00	4.66	2.85	2.78	+	+
34	7.48	5.88	4.88	5.40	3.66	4.08	+	+
35	7.11	4.34	5.32	4.97	3.26	3.84	-	-
36	4.48	<2	3.00	3.00	2.00	2.30	-	-
37	6.27	4.00	4.19	4.42	2.30	2.90	-	-
38	4.62	3.95	<2	<2	<2	<2	-	-
39	7.33	5.00	5.44	4.48	2.26	5.30	+	+
40	7.00	5.04	5.20	4.24	<2	3.00	-	-
41	7.42	5.36	4.48	6.70	1.60	4.00	+	-
42	5.00	<2	2.36	4.00	1.74	4.00	+	+
43	7.41	5.82	5.35	5.03	2.00	4.30	+	+
44	6.33	4.62	5.15	3.00	3.20	3.30	+	+
45	5.29	3.54	2.60	3.78	3.20	4.28	-	-
46	6.72	3.30	3.00	4.66	4.15	4.72	-	-
47	6.30	3.00	3.55	4.30	<2	3.48	+	+
48	7.22	4.48	4.88	5.64	3.95	4.30	-	-
49	8.18	3.25	3.30	4.60	3.85	<2	+	+
50	7.72	4.91	4.48	6.08	5.60	2.95	-	-
51	6.46	5.10	4.63	5.68	2.70	3.00	-	-
52	6.10	4.23	4.48	2.72	<2	<2	+	+
53	6.26	4.82	4.30	3.99	1.60	6.06	+	+
54	7.02	5.25	5.18	2.90	<2	3.90	+	-
55	7.09	5.90	5.67	4.32	3.08	<2	+	-
56	7.18	5.03	5.20	5.37	2.40	3.48	-	-
57	6.22	6.34	3.88	5.33	3.60	4.26	+	+
58	5.82	2.64	5.48	4.35	<2	4.99	+	-
59	5.92	5.98	4.54	3.62	<2	<2	-	-
60	7.11	3.68	3.36	5.66	3.88	4.38	-	-
61	4.00	2.48	2.90	4.23	3.32	3.49	-	-
62	9.52	7.23	5.20	6.76	0.00	4.30	-	-
63	5.75	5.66	5.66	<2	5.67	4.00	-	-
64	7.23	5.26	4.77	5.60	3.36	3.76	-	+
65	7.87	5.82	5.97	<2	3.87	4.27	-	+
66	7.16	3.90	4.95	6.20	4.03	4.51	+	+
67	6.72	3.69	4.23	5.12	3.00	3.11	+	+
68	5.70	5.20	5.30	5.75	3.65	<2	+	+
69	7.24	4.00	4.70	5.42	3.20	<2	+	+
70	4.88	4.16	4.00	4.00	<2	3.87	+	+
71	6.30	2.85	5.33	5.08	2.70	3.00	-	-
72	5.59	4.13	4.34	5.29	3.23	3.71	+	+

As shown in Table 1, the total aerobic mesophilic bacteria count (TAMB), *Pseudomonas*, yeast and moulds, coliform bacteria, *Enterococcus* and *Staphylococcus*/*Micrococcus* counts of the 72 chicken meat samples showed differences of 4.00-10.05, <10-7.23, <10-5.97, <10-7.61, <10-5.67 and <10-6.06, respectively.

Thirty of the 72 (41.67%) chicken meat samples were positive for *Salmonella* spp. by the conventional method, whereas 42 of the 72 (58.33%) samples were positive by the IMS-PCR method. Thirty of the 72 (41.67%) samples were positive for *Salmonella* spp. by both methods (Table 2).

Table 2. The obtained *Salmonella* spp. results that were determined by conventional and IMS-PCR methods.

	Conventional	IMS/PCR	Both methods
n/N	30/72	42/72	30/72
%	41.67	58.33	41.67

The IMS-PCR technique was applied to the chicken meat samples collected from different markets and retail stores. The *invA* (284 bp) gene was prepared using the IMS-PCR technique. The *invA*FW: 5′-ACA GTG CTC GTT TAC GAC CTG AAT-3′ and *invA*RV 5′-AGA CGA CTG GTA CTG ATC GAT AAT-3′ primers were used to specifically amplify a 284-bp genomic fragment of the *invA* gene. The PCR image is shown in Fig. 1. As shown in Fig. 1, lane 9 was the negative control and lane 8 was the positive control, whereas lanes 2-7 were the *Salmonella* spp. positive samples. Lane 1 was verified to be a *Salmonella* spp.-negative sample. All *Salmonella* spp. determined by the conventional method carried the *invA* gene region.

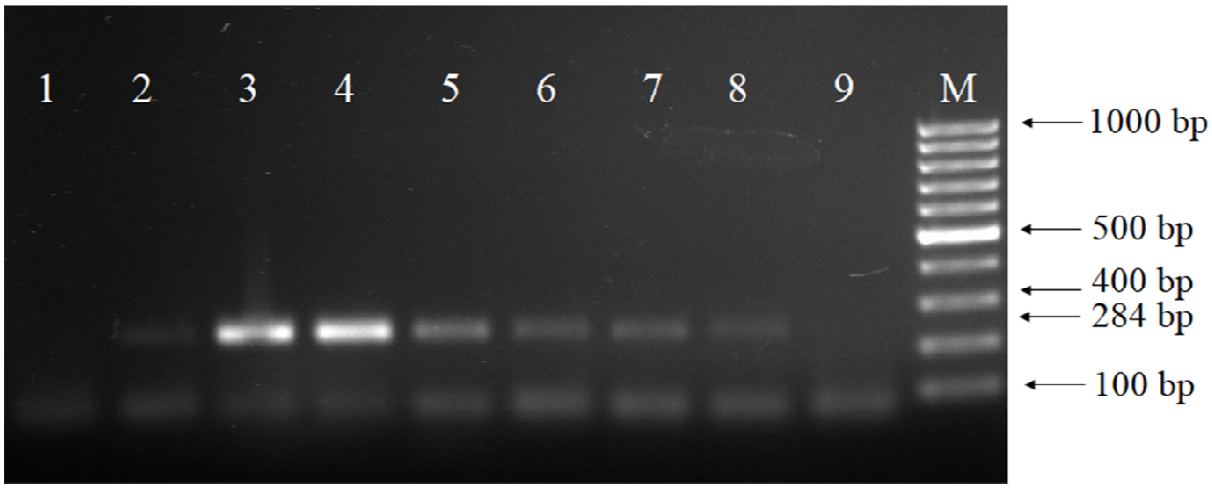

Figure 1. Detection of the invA (284 bp) gene by immunomagnetic separation-polymerase chain reaction. The samples were electrophoresed on a 1% agarose gel. M: 1,000 bp marker; Lane 9: Negative control; Lane 8: Positive control (*Salmonella typhimurium* RSSK 95091; Lanes 2–7: *Salmonella* spp. positive samples; Lane 1: *Salmonella* spp. negative sample).

The antibiotic resistance and susceptibility of 30 *Salmonella* spp. isolates were determined using 10 different antibiotics (Table 3). As shown in Table 3, 27 of the 30 isolates showed the highest susceptibility to CN, while all and 28 of the 30 isolates had the highest resistance to NA and TE, respectively. The highest susceptibility occurred in response to CN, followed by C, AMP, K, SXT, W, S, CIP, TE and NA, respectively. In contrast, the highest resistance was to NA and TE, followed by W, S, SXT, K, AMP, C, CIP and CN, respectively

Isolates that are resistant to three or more antibiotics are classified as multidrug resistant (MDR). It was determined that all [30 (100%)] isolates were resistant to at least one antimicrobial agent, whereas 25 (83.33%) were MDR. About 47% of the isolates showed resistance to five antibiotics, while 13.33% were resistant to six antibiotics. The distribution of antimicrobial resistance characteristics of the *Salmonella* spp. isolates is presented in Table 4.

Table 3. Antibiotic susceptibility and resistance of obtained isolates (N:30).

Antibiotics	Susceptible		Intermediate		Resistant	
	%	N	%	N	%	N
Streptomycin (S)	6.67	2	23.33	7	70.00	21
Nalidixic acid (NA)	0	0	0	0	100	30
Ciprofloxacin (CIP)	3.33	1	90.00	27	6.67	2
Trimethoprim (W)	20.00	6	6.67	2	73.33	22
Chloramphenicol (C)	73.33	22	6.67	2	20.00	6
Gentamicin (CN)	90	27	6.67	2	3.33	1
Trimethoprim/Sulfamethoxazole (SXT)	23.33	7	10.00	3	66.67	20
Ampicilin (AMP)	66.67	20	6.67	2	26.67	8
Kanamycin (K)	43.33	13	10.00	3	46.67	14
Tetracycline (TE)	3.33	1	3.33	1	93.34	28

Table 4. The distrubiton of antimicrobial resistance characteristics of *Salmonella* spp. isolates.

One type of antimicrobial	NA	1 (3.33%)
Two types of antimicrobials	NA, TE	3 (10.00%)
	SXT, TE	1 (3.33%)
Three types of antimicrobials	NA, SXT, TE	1 (3.33%)
	NA, W, TE	1 (3.33%)
Four types of antimicrobials	NA, CIP, AMP, TE	1 (3.33%)
	NA, W, SXT, TE	1 (3.33%)
	NA, W, C, TE	1 (3.33%)
	NA, W, SXT, TE	2 (6.67%)
Five types of antimicrobials	S, NA, W, AMP, K	1 (3.33%)
	S, NA, W, SXT, TE	1 (3.33%)
	NA, W, SXT, K, TE	2 (6.67%)
	NA, W, SXT, AMP, TE	2 (6.67%)
	NA, C, AMP, K, TE	2 (6.67%)
	NA, W, SXT, K, TE	6 (20.00%)
Six types of antimicrobials	NA, W, C, SXT, AMP, TE	1 (3.33%)
	NA, W, C, SXT, K, TE	1 (3.33%)
	NA, W, CN, SXT, K, TE	1 (3.33%)
	W, C, SXT, AMP, K, TE	1 (3.33%)
		30 (100%)

DISCUSSION

The general microbiological quality parameters of the samples were determined with respect to TAMB, *Pseudomonas*, yeast and mould, coliform bacteria, *Enterococcus* and *Staphylococcus*/*Micrococcus* counts. According to the Food Standard, the presence of *Salmonella* spp., coliform and other bacteria are accepted as the hygiene index (ISO 2001, 2002). However, the results obtained here show that the samples did not meet the standards with respect to the presence of microorganisms. High numbers of bacteria shorten shelf life by deteriorating meat quality, resulting in an economic loss. Our results seem to be due to processing and storage conditions as well as cross-contamination after processing in the markets and homes.

The increase in food contamination factors and gastrointestinal diseases is associated with an increase in the risk of non-specific salmonellosis (CRUM-CIANFLONE, 2008). In Turkey and most developing countries, the absence of epidemiological studies of salmonellosis cases is an

obstacle to effectively assess prevalence (KAFERSTEIN, 2003).

Our data corroborate previous studies revealing that chicken meat is an important food source contaminated by *Salmonella* spp. The difference in prevalence data for *Salmonella* spp. between previous studies and the present study might be due to sanitation conditions, methodological differences used to isolate the bacteria or transportation and storage conditions (LI et al., 2013). In this study, *Salmonella* spp. was detected in 41.67% of the chicken meat samples using the conventional method and 58.33% of the samples using the IMS-PCR technique. According to the Turkish Food Codex Communiqué on Microbiological Criteria, *Salmonella* spp. should not be found in any meat (TFC, 2011). Our results were lower than those reported previously (150/64, 42.66%) by Siriken et al. (2015) in Ankara, Turkey. However, Yildirim et al. (2011) reported a 34% (200/68) contamination rate by *Salmonella* in poultry meat in Turkey. The contamination rate of *Salmonella* in poultry meat varies among countries (ALVAREZ-FERNANDEZ et al., 2012). In the present study, the high prevalence rate of *Salmonella* in poultry meat was similar to the 60% reported in Portugal (ANTUNES et al., 2003) and 67.5% in Thailand (LERTWORAPREECHA et al., 2012), and was close to the contamination rates of 55% in Spain and 52.2% in China (YANG et al., 2011). In contrast, the incidence rates of *Salmonella* in poultry meat in South Korea and Pakistan were 3.7% (RAN HEE et al., 2014) and 5.26% (AKBAR; ANAL, 2013), respectively. These differences might be due to geographical location, bacteriological analytical methods, the sampling pattern or factors, such as hygiene and sanitation conditions during poultry meat production, cross-contamination or market conditions. Although the prevalence of *Salmonella* determined by the conventional method was close to or lower than that reported by many studies, it was possible to detect *Salmonella* using the IMS-PCR technique. All of these results indicate that poultry meat is an important source for *Salmonella* spp. infections.

The results show that the IMS-PCR technique was superior to the conventional method. The superiority of IMS-PCR over the conventional method might be due to the concentrations of the target microorganism in the samples, removal of inhibitor components or elimination of other microorganisms. Similar results were reported by Siriken et al. (2015) who detected *Salmonella* spp. in beef and poultry meat by both conventional and IMS methods. The IMS-PCR technique allows rapid detection of *Salmonella* spp. after IMS. Zheng et al. (2016) reported that IMS-PCR has an accuracy of 98.3%.

The present study is significant because it is the first report that has detected the *invA* gene region of *Salmonella* in chicken meat. The PCR products of the isolates contained a PCR-positive control, which resulted in detection of a 284 bp amplified fragment. The ability of *Salmonella*-specific primers to detect *Salmonella* spp. rapidly and accurately was primarily due to the primer sequences selected from the *invA* gene. The *invA* protein in the inner membrane of *Salmonella* spp. is required for invasion of the bacterium into epithelial cells (SHARMA; DAS, 2016). All *Salmonella* spp. identified by conventional methods carry the *invA* gene region. Furthermore, this genus invades intestinal epithelial cells and the gene is found in pathogenic *Salmonella* spp. Therefore, it is important to identify the gene responsible for invasion. The *invA* gene is thought to trigger the internalisation necessary for invasion to deeper tissues, which is necessary for complete virulence of *Salmonella* spp. (LEI et al., 2015).

The resistance of bacteria to antibiotics has global importance in terms of failed zoonotic disease treatment. Antibiotic-resistant bacteria are important to public health, and their resistance genes are consumed with contaminated food or water (HONG et al., 2013). The most important source of antibiotic-resistant *Salmonella* spp. is animal-originating foods. The data obtained from this study and other studies support these claims.

In this study, 10 different antibiotics were tested to determine antibiotic resistance of the isolates. Among the antibiotics, CN had the greatest effect on the isolates, followed by C, AMP, K, SXT, W, S, CIP, TE and NA. Cetinkaya et al. (2008) reported that *Salmonella* spp. was detectable in only one poultry meat sample in a study on the presence of *Salmonella* in different food samples (chicken parts, minced meat, ready-to-eat salad, raw vegetables and raw milk) sold in Bursa, Turkey. They identified the *Salmonella* spp. isolated as *S. infantis* and reported resistance to streptomycin, tetracycline, sulphonamides, trimethoprim, trimethoprim-sulphamethoxazole and NA. In another study, Kasimoglu Dogru et al. (2010) reported that 22 (68.75%) of 32 strains isolated from poultry meat were resistant to multiple antibiotics. According to the same study, *Salmonella* spp. was most resistant to NA (62.5%). Siriken et al. (2015) reported that *Salmonella* strains isolated from different meat samples are most resistant to vancomycin, tetracycline, streptomycin and NA. In

the present study, the highest resistance of *Salmonella* spp. was to tetracycline and NA (100 and 93.34%, respectively). In contrast, Arslan and Eyi (2010) reported that 50 of 225 meat samples were positive for *Salmonella* spp. These isolates had the highest resistance to ampicillin and cephazoline. It was also suggested that 62% of *Salmonella* strains have multiple resistance to tetracycline, carbenicillin, ampicillin and sulfamethoxazole-trimethoprim.

The high resistance to trimethoprime and sulfamethoxazole-trimethoprime was not surprising due to their continued use in human and veterinary clinics in Turkey. In addition, resistance to streptomycin was significantly higher than that reported in previous studies (LESTARI et al., 2009; MOLLA et al., 2003). Other studies have shown that resistance to streptomycin is due to its high prevalence and frequent use in veterinary medicine (MIHAIU et al., 2014). A higher resistance to streptomycin (95%) was also reported by White et al. (2001) for *Salmonella* spp. isolated from chicken meat.

In the present study, *Salmonella* spp. isolated from chicken meat parts was highly resistant to NA (100%). Our findings are consistent with studies conducted in different countries (CUI et al., 2016; SODAGARI et al., 2015) and in Turkey (KASIMOGLU DOGRU et al., 2010; SIRIKEN et al., 2015). In the past, ampicillin, chloramphenicol and co-trimoxazole were used to treat salmonellosis, but they have been replaced by fluoroquinolone antibiotics, such as ciprofloxacin and cephalosporin. After tetracycline, fluoroquinolones are the most widely used antibiotic group in veterinary medicine and are expected to become more resistant. NA resistance plays a role in the first steps of the development of ciprofloxacin resistance, although low resistance (6.67%) of ciprofloxacin resistance is observed in the isolates. It should be emphasised that third-generation cephalosporins have recently become the primary drug to treat salmonellosis cases due to an increase in fluoroquinolone resistance (MAWATARI et al., 2013). For this reason, resistance to fluoroquinolones has emerged as an important public health issue because these antibiotics, which are widely used in veterinary medicine and poultry production, can cause resistance genes to be transmitted to humans through the food chain (GONZÁLEZ; ARAQUE, 2013).

In the present study, 26.67% of the isolates were resistant to ampicillin, which was remarkably lower than the resistance reported in other countries by Thung et al. (2016) in Malaysia (72.73%), Trongjit et al. (2017) in Thailand (72.4%) and Yen et al. (2014) in Vietnam (41.6%). It is not surprising that ampicillin is still preferred in the classical treatment of salmonellosis in humans and it is not often used in animal therapy in Turkey.

According to a report published by EFSA in 2014 (EFSA, 2014), *Salmonella* spp. isolates of broiler origin in 22 different European Union countries were reported to have the highest resistance to NA (48.7%), sulfamethoxazole (45.1%) and tetracycline (40.4%). Although the results obtained in our study excluding resistance to trimethoprim were qualitatively similar, resistance rates were determined to be higher in our study. However, these results are similar to those obtained from countries, such as Bulgaria and Hungary, as they reflect the average of the data of 22 different member countries. The low level of resistance to gentamicin (6.6%) was close to our study results (4.76%). In the same report, resistance to chloramphenicol was lower (4%), although it was high in the present study (26.98%).

CONCLUSIONS

The results showed that 30 of the 72 samples were positive for *Salmonella* spp. by the conventional method, while 42 of the 72 were positive by IMS-PCR. In contrast, 30 of the 72 samples were positive by both methods. In conclusion, combining the IMS and PCR methods was used effectively to isolate *Salmonella* from chicken than either method alone.

The 30 identified *Salmonella* spp. isolates were evaluated in terms of antibiotic resistance and susceptibility. The results indicated that the highest resistance was to NA and TE, while the highest susceptibility was to GN. Taken together, it is evident that chicken meat is a serious public health risk in this region in terms of the presence of *Salmonella* spp. in chicken meat parts, antibiotic resistance of isolates and microbiological properties of chicken meat parts.

RESUMO: Parâmetros microbiológicos específicos e a presença de *Salmonella* spp. foram investigados em 72 amostras de carne de frango (36 asas e 36 baquetas) coletadas em mercados e açougues. Os parâmetros microbiológicos específicos foram determinados utilizando um método cultural convencional e a presença de *Salmonella* spp. em amostras de frango foi determinada utilizando métodos de reação em cadeia da

polimerase (PCR) por separação convencional e imunomagnética (IMS). Além disso, a suscetibilidade antimicrobiana dos isolados foi revelada pelo método de difusão do disco de Kirby-Bauer. Os resultados indicaram que 30 das 72 amostras foram positivas para *Salmonella* spp. pelo método convencional, e 42 das 72 foram positivas pelo método IMS-PCR. No entanto, 30 das 72 amostras foram positivas para *Salmonella* spp. por ambos os métodos. Os isolados de *Salmonella* spp. foram confirmados pelo sistema VITEK2 Compact e PCR. As susceptibilidades dos isolados a 10 antibióticos foram determinadas. Os resultados indicaram que os isolados (27/30) apresentaram maior suscetibilidade à gentamicina (90,00%), enquanto a maior resistência foi ao ácido nalidíxico e à tetraciclina nos níveis de 100 e 93,34%, respectivamente. Estes resultados indicam uma alta prevalência de *Salmonella* spp. em carne de frango da cidade de Erzurum, Turquia, e o perfil de resistência antimicrobiana desses isolados deve ser considerado para a saúde pública. Os resultados também demonstram que a técnica de IMS-PCR foi superior ao método convencional para detecção de *Salmonella* em carne de frango.

PALAVRAS-CHAVE: Carne de frango. *Salmonella.* IMS. PCR. Antimicrobiano.

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