

DETERMINATION OF MICROBIOLOGICAL QUALITY AND PRESENCE OF *SALMONELLA* SPP. ON CHICKEN PARTS SOLD AT RETAIL MARKETS IN ERZURUM, ANTIBIOTIC RESISTANCE OF THE *SALMONELLA* SPP. ISOLATES

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ABSTRACT

The present study was conducted to investigate the certain microbiological quality characteristics, and presence of *Salmonella* spp. by immunomagnetic separation (IMS) and conventional method, also to evaluate the antibiotic resistance of *Salmonella* spp. isolates obtained from 45 chicken meat samples (fifteen livers, chests, baguettes) collected from markets at the Erzurum. Totally, in the 15 samples were found positive for *Salmonella* spp. by IMS and conventional methods. Isolates were identified with 99.9% probability using API 20E biochemical analysis kit. The results of antibiotic test showed the susceptibility of the isolates to ciprofloxacin, chloramphenicol and gentamicin at 100% level, while these isolates were resistance to tetracycline, trimethoprim, sulfamethoxazole/trimethoprim, nalidixic acid, streptomycin, ampicillin and kanamycin, respectively. The obtained results showed that 84% (38/45) of the chicken meat samples did not show suitability with Turkish Food Codex in terms of total bacteria count and presence of *Salmonella* in 33% (15/45) of the samples.

Keywords: Chicken meats, microbiological quality, *Salmonella* spp., immunomagnetic separation (IMS), antibiotic resistance

ERZURUM'DA SATIŞA SUNULAN TAVUK ETLERİNİN MİKROBİYOLOJİK KALİTESİ VE *SALMONELLA* SPP. VARLIĞININ BELİRLENMESİ, *SALMONELLA* SPP. İZOLATLARIN ANTİBİYOTİK DİRENCİ

ÖZ

Bu çalışma, Erzurum'da marketlerden toplanan 45 adet tavuk eti örneğinde (15'er ciğer, göğüs, bagnet) belirli mikrobiyolojik kalite karakteristikleri, immunomanyetik separasyon (IMS) ve geleneksel yöntem ile *Salmonella* spp. varlığını araştırmak için yapılmıştır. Ayrıca, *Salmonella* spp. izolatlarının antibiyotik dirençleri değerlendirilmiştir. Toplamda 15 örnekte IMS ve geleneksel metot ile *Salmonella* spp. pozitif bulunmuştur. Bu izolatlar API 20E biyokimyasal analiz kiti kullanılarak %99,9 ihtimalle doğrulanmıştır. *Salmonella* spp. izolatları siprofloksasin, kloromfenikol ve gentamisin antibiyotiklerine karşı %100 seviyesinde duyarlılık gösterirken, sırasıyla tetrasiklin, trimetoprim, sülfametoksazol/trimetoprim, nalidiksik asit, streptomisin, ampisilin ve kanamisin antibiyotiklerine karşı direnç göstermiştir. Elde edilen sonuçlara göre tavuk etlerinin %84'ü (38/45) toplam bakteri sayısı, %33'ünün (15/45) *Salmonella* spp. varlığı açısından Türk Gıda Kodeksi'ne uygun olmadığı görülmüştür.

Anahtar kelimeler: Tavuk etleri, mikrobiyolojik kalite, *Salmonella* spp., immunomanyetik separasyon, antibiyotik direnç

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INTRODUCTION

Chicken meats can be contaminated with certain pathogen bacteria and various microorganisms. Raw chicken meats may harbour many important pathogenic microbes i.e. *Salmonella* spp., *Campylobacter jejuni/coli*, *Yersinia enterocolitica*, *E. coli*, *S. aureus* and *Listeria monocytogenes*, causing risks in meat products for human health, under the poor hygiene conditions (Sharma & Chattopadhyay, 2015). Among these microorganisms, *Salmonella* spp. is the major agent. Transmission of many potential microorganisms may occur during the processing of chicken. For that reason, chicken meats can be thought as one of the major food vehicles for this pathogen. Improper processing conditions can cause cross contamination from beforehand handled raw chicken carcasses and parts to cooked chicken meats. Consumption of contaminated raw or undercooked poultry products (particularly chicken meat) is the primary reason of *Salmonella* infections in humans (Bryan & Doyle, 1995; Çetin, 2006; Park et al., 2014).

Salmonella is the most frequently reported pathogen among causative agents of foodborne diseases worldwide. Moreover, antimicrobial resistance can be considered as a growing problem in terms of poultry-associated pathogens. As a result of using antibiotics for therapeutic, prophylactic and non-therapeutic objectives in commercial poultry meat production, antibiotic-resistant and multi-drug resistant strains of *Salmonella* can be found in the final product. The emergence of antibiotic resistance of bacterial agents has become a major public health concern (Salihu et al., 2014; Sapkota et al., 2014; Gurler et al., 2015).

Ensuring the safety of poultry meats by early detection of foodborne pathogens would be regarded as a main factor in preventing *Salmonella* contamination. The controlling of poultry and other related products for *Salmonella* contamination can be made particularly more efficient by using quick and sensible determination methods (Park et al., 2014).

In immunomagnetic separation (IMS) system, superparamagnetic beads coated with antibodies

are used against surface antigens of the cells for the efficient isolation of target bacteria. Isolation of target pathogen bound to the beads with the antigen-antibody interaction can be determined by transferring the inoculated bead samples to culture broths or selective agar for the microorganism. Immunomagnetic separation (IMS) is successfully used to step-down the enrichment stage for reducing the total analysis as much as 24 h. In this method, super paramagnetic beads or polystyrene particles are used as coated with iron oxides or oxide-hydroxides. The antibodies allow for the specific holding and isolation of pathogen microorganisms from the present microflora of food samples (Taban & Ayaç, 2009; Foddai et al., 2010; Wadud et al., 2010; Chakraborty et al., 2011).

The aim of this research was to evaluate the changes in certain microbiological properties (total aerobic mesophilic bacteria (TAMB), coliform bacteria, psychrotrophic bacteria, yeast-mould and *Enterococcus* spp.) and the presence of *Salmonella* spp. in different parts of chicken (liver, chest and baguette) obtained from markets in the Erzurum. For the determination of the *Salmonella* existence in the chicken meats by conventional and IMS methods., Another aim of the study is to show the antibiotic susceptibility and resistance of identified *Salmonella* spp. isolates against 10 different types of antibiotic.

MATERIALS AND METHODS

Materials

Totally 45 fresh chicken meat samples (15 livers, 15 chests and 15 baguettes,) were taken from different markets and butcher shops in the Erzurum. The samples were immediately transported to laboratory under cold conditions and kept at 4°C for maximum 1 h before analysis.

General microbiological analysis

For liver and chest samples, 25 g were weighted and diluted aseptically in 225 mL sterile maximum recovery diluent (MRD) (0.85% NaCl+0.1% peptone) and homogenised in filtered polyethylene bag using a Stomacher (Seward Laboratory Blender Stomacher 400 Lab Blender, UK) for 5 min. However, one baguette was used

for each sample and rinsed for 2 min in a sterile filter stomacher bag containing 500 mL sterile MRD. Then serial decimal dilutions of homogenates were prepared and plated on specific media. Total aerobic mesophilic bacteria count were determined on Plate Count Agar (PCA) (Merck, Darmstadt, Germany) at $30\pm 1^\circ\text{C}$ for 48 h (Harrigan, 1998; Maturin & Peeler, 1998; ISO, 2013). Total coliform counts were determined using Violet Red Bile Agar (VRB) (Merck, Darmstadt, Germany) at $37\pm 1^\circ\text{C}$ for 48 h (Harrigan, 1998; ISO, 2006). The counts of psychotropic bacteria were enumerated on PCA at $7-10 \pm 1^\circ\text{C}$ for 10 days (ISO, 2001), and yeast-moulds were determined on Potato Dextrose Agar (PDA) (Merck, Darmstadt, Germany) acidified with 10% lactic acid (Merck, Darmstadt, Germany) and incubated at $25\pm 1^\circ\text{C}$ for 5-7 days (Koburger & Marth, 1984; ISO, 2008). Selective enumeration and detection of *Enterococcus* spp. were performed on Kanamycin Aesculin Azide Agar (KEAA) (Oxoid, Hampshire, UK). The plates of *Enterococcus* spp. cultures were incubated under aerobic conditions at $35-37\pm 1^\circ\text{C}$ for 24 h (Harrigan, 1998; ISO, 2000; Sanlibaba et al., 2018).

Bacterial strain

Salmonella Typhimurium RSSK 95091 were supplied by the Refik Saydam Hıfzıssıhha Culture Collection (Turkey).

Isolation and Identification Protocols of *Salmonella*

Samples were analysed according to ISO 6579-1:2017 (Microbiology of the food chain-horizontal method for the detection, enumeration and serotyping of *Salmonella*-part 1: detection of *Salmonella* spp.) for the detection of *Salmonella* (Yüksel Kavaz & Yüksel, 2015; ISO, 2017). For each chicken liver and chest sample, 25 g was pre-enriched in 225 mL Buffered Peptone Water (Oxoid, Hampshire, UK) at 37°C for 24 h. Chicken baguettes were rinsed for 2 min in a sterile filter stomacher bag containing 500 mL sterile distilled water and then 25 mL was pre-enriched in 225 mL Buffered Peptone Water. Afterwards, 0.1 mL of pre-enrichment samples were transferred in 9.9 mL of Rappaport-

Vassiliadis (RVS) Enrichment Broth (Oxoid, Hampshire, UK) and 1 ml in 9 ml of Muller-Kauffmann Tetrathionate Novobiocine enrichment broth (MKTTn) (Merck, Darmstadt, Germany) and tubes were incubated at $42\pm 1^\circ\text{C}$ for 24 h for RVS, at $37\pm 1^\circ\text{C}$ for 24 h for MKTTn, respectively. After overnight incubation, a loopful of the enrichment samples was streaked onto Xylose Lysine Deoxycholate Agar (XLD) (Merck, Darmstadt, Germany) and Xylose Lysine Tergitol-4 Agar Base (XLT4) (Merck, Darmstadt, Germany). Both selective agar media were incubated for 24 h at 37°C

IMS was performed according to the manufacturer's protocol (Invitrogen, California, USA). Twenty micro liter for *Salmonella* spp. Dynabeads anti-*Salmonella* (Invitrogen, California, USA) were incubated with 1 mL of the pre-enriched BPW of each sample in 2 mL micro-centrifuge tube at room temperature for 15 min with repeated rocking, so that the specific antibodies coated on to the beads would bind *Salmonella*. The bead-bacteria complex were subsequently separated using a magnetic particle collector (Dynamag; Invitrogen, California, USA). Afterwards, 1 mL of washing buffer (PBS (Oxoid, Hampshire, UK), and 0.05% Tween 20 (Merck, Darmstadt, Germany) was added to resuspend the beads and were washed three times with PBS (pH 7.4) solution. Finally, 200 μL of washing buffer was added to resuspend the beads. Afterwards, equally 100 μL of the complex was plated onto XLD and XLT4 Agar. The selective agars media were incubated for 24 h at 37°C .

Identification

After isolation of *Salmonella* by both IMS and conventional method, suspicious colonies were tested by Gram staining and oxidase reaction. Both Gram-negative and Oxidase-negative isolates were further tested. Then, isolated colonies were transferred to tubes with Triple Sugar Iron agar (Oxoid, Hampshire, UK) and Lysine Iron agar (Oxoid, Hampshire, UK), and incubated at $35-37^\circ\text{C}$ for 18-24 h. Additional biochemical tests were performed by using API 20E test kit (bioMérieux, Marcy-l'Étoile, France). The plastic strips holding twenty mini-test tubes

were inoculated with the saline suspensions of the cultures according to manufacturer's directions. After incubation in a humidity chamber for 18-24 hours at 37°C, the colour reactions were read (some with the aid of added reagents as supplied by the kit). The data were analysed by the manufacturer's software (apiweb) and positive results with $\geq 99.9\%$ probabilities were confirmed as *Salmonella* spp.

Antibiotic resistance testing

The antibiotic resistance profiles of isolated *Salmonella* spp. were determined with Kirby-Bauer Disk diffusion protocol recommended as the standard of Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2014). A loopfull of each pure bacterial isolate was emulsified in 5 mL of sterile 0.85% NaCl solutions, and the density was compared with a barium chloride (BaCl₂) standard (0.5 McFarland). A sterile cotton swab was dipped into the standardized suspension of bacterial cultures and used to evenly inoculate the Mueller-Hinton Agar plates (Oxoid, Hampshire, UK), and the plates were allowed to dry. Antibiotic discs with the following drug contents ciprofloxacin (CIP, 5 µg), sulfamethox/trimethoprim (SXT, 5 µg), chloramphenicol (C, 30 µg), streptomycin (S, 10 µg), ampicillin (AMP, 10 µg), gentamicin (CN, 10 µg), kanamycin (K, 30 µg), nalidixic acid (NA, 30 µg), trimethoprim (W, 5 µg) and tetracycline (TE, 30 µg) (supplied by Oxoid, Hampshire, UK) were placed at least 15 mm apart and from the edge of the plates to prevent the overlapping of the inhibition zones. Plates were incubated at 37°C for 24 h, and the diameters of zones of inhibition were measured with a ruler. The sizes of the inhibition zones allowed the strains to be classified as susceptible and resistant according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2014).

Storage of the isolates

Positive colonies were streaked onto Nutrient Agar (NA) and incubated overnight at 37°C. A generous colony swab was collected from each NA plate and stored at -80°C in with 15% glycerol.

RESULTS AND DISCUSSION

General microbiological quality characteristics of chicken meat samples

The general microbiological composition of chicken meat samples and mean values are shown in Table 1 (Log CFU/g).

Observing the certain microbiological quality characteristics of chicken meat samples, the lowest mean counts of TAMB and coliform bacteria were found in L14 sample. The highest mean values of TAMB count were determined in B12 sample, while the highest coliform bacteria were detected in sample B15 (Table 1). Similar TAMB results were also reported by Çetin (2006). As seen in Table 1, the mean psychrotrophic bacteria counts of chicken meats were between 5.00 Log CFU/g (Sample C7) to 8.67 Log CFU/g (Sample B9). Similar findings were reported by Çetin (2006). The highest yeast and mould counts were determined in sample B9, while the lowest mean value was in sample L11. Çetin (2006) reported that the number of yeast and mould of the observed chicken meats was found as 5-6 Log CFU/g. *Enterococcus* spp. was not determined in 10 liver samples (Samples L1, L3, L5, L6, L7, L8, L10, L11, L13, L15), 9 chest samples (Samples C1, C2, C3, C5, C7, C8, C11, C12, C14) and 8 baguette samples (B1, B3, B6, B9, B11, B12, B14 and B15). Based on these results, it might be said that baguette samples had highest microbiological load in terms of investigated microorganisms, followed by liver and chest samples. According to the Turkish Food Codex it is allowed to have raw poultry meat less than 5.0 Log CFU/g of total aerobic mesophilic bacteria (Anonymous, 2009). The obtained results showed that 84% (38/45) of the observed chicken meat samples did not show suitability with these standards in terms of TAMB count and similarly, 33% (15/45) of the samples was not suitable with respect to the presence of *Salmonella*. High microbiological counts in the samples is thought to be stemmed from the process of cutting, plucking, washing, cooling, freezing and storage.

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Table 1. The certain viable bacteria counts of the chicken meat samples (Log CFU/g)

Samples	TAMB count	Coliform bacteria	Psychrotrophic bacteria count	Yeast-Mould	<i>Enterococcus</i> spp.
LIVERS (Log CFU/g)					
L1	7.69	3.39	6.04	6.17	<10
L2	7.47	3.54	5.60	6.30	2.47
L3	7.00	3.30	6.77	6.39	<10
L4	7.47	3.20	5.69	6.54	2.60
L5	6.85	2.47	6.34	6.00	<10
L6	7.54	3.00	6.84	5.95	<10
L7	7.00	4.12	6.91	6.17	<10
L8	6.45	3.26	5.74	6.23	<10
L9	7.30	3.55	6.52	6.74	3.00
L10	7.54	3.00	6.60	6.23	<10
L11	7.35	3.12	5.84	5.50	<10
L12	6.35	2.78	6.12	6.12	2.55
L13	7.00	2.84	5.32	5.45	<10
L14	5.85	2.25	6.53	6.45	2.20
L15	7.54	3.10	5.91	5.92	<10
CHESTS					
C1	7.01	3.23	6.35	6.49	<10
C2	7.44	3.11	6.83	6.46	<10
C3	7.45	3.27	5.65	5.95	<10
C4	6.35	2.90	6.68	6.45	2.74
C5	7.39	3.20	6.42	6.50	<10
C6	7.43	3.39	6.32	6.29	2.69
C7	6.85	3.22	5.00	6.10	<10
C8	5.95	3.10	5.24	6.23	<10
C9	7.32	3.47	6.10	6.78	2.84
C10	6.86	2.90	6.51	6.12	2.20
C11	6.40	2.65	6.55	5.90	<10
C12	7.40	3.25	6.32	6.46	<10
C13	7.21	3.10	6.65	6.19	2.00
C14	6.35	3.45	5.95	6.43	<10
C15	7.30	3.85	5.35	6.62	2.92
BAGUETTE					
B1	7.70	5.69	8.47	7.47	<10
B2	8.81	5.25	8.27	7.30	4.88
B3	7.60	5.55	8.43	7.55	<10
B4	8.76	5.61	8.14	6.51	4.94
B5	8.96	5.74	7.80	7.64	4.74
B6	7.43	5.87	8.34	7.01	<10
B7	8.45	4.81	8.14	7.30	3.98
B8	7.69	5.94	7.92	7.48	4.20
B9	9.01	5.67	8.67	7.69	<10
B10	8.85	5.32	8.32	7.64	4.58
B11	8.92	5.78	8.20	6.65	<10
B12	9.60	5.81	7.92	7.10	<10
B13	8.54	5.98	8.55	7.50	4.87
B14	7.12	5.67	7.44	7.30	<10
B15	9.01	6.00	8.12	6.40	<10

The suspicious *Salmonella* spp. colonies were identified with API 20E at the probability of 99.9%. Consequently, the results demonstrated that conventional and IMS methods showed similarity approximately with regard to the identification of *Salmonella* spp. in chicken meat samples.

The 15 chicken meat samples were found positive in terms of *Salmonella* by conventional method, while 13 the samples were determined as positive by IMS method (Table 2). *Salmonella* spp. were found positive in two samples by conventional method but could not be detect by IMS. The presence of *Salmonella* spp. in raw chicken livers, chests and baguettes were at the level of 27%, 33% and 40%, respectively. The chicken baguette was found higher microbial load than chest and liver. The presence of *Salmonella* spp. in investigated chicken meat samples might stem from the unhygienic conditions (Çetin, 2006; Procura et al., 2017; Zwe et al., 2018). Relatively inferior hygiene practices, such as cutting chickens with knife and chopping board without proper cleaning, and exhibiting chicken carcasses in the chillers without physical separation or individual packaging could likely contribute to cross-contamination events leading to a significantly higher rate of *Salmonella* spp. contamination in chicken meats sold in markets of Erzurum.

Table 2. Distribution of IMS with conventional methodology for *Salmonella* spp. detection in chicken meats.

	Conventional	IMS
n/N	15/45	13/45
%	33	28

(n: *Salmonella* positive sample number; N: Total sample number)

Recently, various researches have been focused on the presence of *Salmonella* spp. in poultry meat (Van et al., 2007; Pointon et al., 2008; Yang et al., 2011; Zwe et al., 2018). This study has shown the high of *Salmonella* contamination (33.3%) in chicken meats. Our results are similar to those

reported by Iseri and Erol (2010) 45.8% of turkey meat samples were showed to be contaminated with *Salmonella*. Also, this *Salmonella* prevalence rate of 33.3 % in raw chicken in Erzurum is similar compared to values reported in Anatolia (Yildirim et al., 2011). The presence of *Salmonella* from chicken meats found in this study was different that in previous works (Uyttendaele et al., 1999; Chung et al., 2003; Angkitittrakul et al., 2005; Van et al., 2007; Pointon et al., 2008; Hyeon et al., 2011; Yang et al., 2011). We assert that the difference of *Salmonella* prevalence between reports might be related with hygiene conditions. The unhygienic and improper processing methods might be the causes for higher incidence of salmonellosis (Ramya et al., 2012).

Antibiotic resistance of *Salmonella* spp. isolates

For the determination of antibiotic susceptibility of *Salmonella* spp. isolates, 10 different antibiotics were used (Table 3 and Table 4). As seen in the Table 3, none of the *Salmonella* spp. isolates were resistant to CIP, C and CN, while resistance of isolates changed, as 10 of them to S, 12 of them to AMP, 5 of them NA and SXT, 3 of them to W and 2 of them to TE, respectively. Among the tested antibiotics, K and S had the highest effect on *Salmonella* spp. isolates and it was followed by AMP, NA, SXT, W, TE, CIP, C and CN, respectively. According to statistical evaluations, all samples showed statistical differences ($P < 0.01$) in terms of antibiotic resistances and susceptibilities (Table 4). These findings are in agreement with previous studies on chicken meat from Iran (Dallal et al., 2010; Sodagari et al., 2015), India (Mir et al., 2015), Turkey (Yildirim et al., 2011) and Egypt (Abd-Elghany et al., 2015), Iraq (Harb et al., 2018).

The results showed that 100% of the isolates were susceptible to CIP, C and CN, while 93.7% of them showed resistance to S and K.

In this research, 45 chicken meat samples (15 livers, 15 chests and 15 baguettes) were analysed with respect to certain microbiological parameters and presence of *Salmonella* spp. The obtained results showed that 84% (38/45) of the observed

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chicken meat samples were not suitable according to the standards with respect to TAMC count and presence of *Salmonella* spp. (33%). In light of the obtained data, baguette samples were found higher microbial load than liver and chest samples in terms of analysed parameters. Then, suspicious colonies of *Salmonella* were confirmed as *Salmonella* spp. at the level of 99.9% confidence interval by API 20E test kit and conventional biochemical tests.

The obtained findings showed that conventional and IMS methods demonstrated similar properties in terms of the determination of *Salmonella* spp. from observed samples. In conclusion, combination of IMS and conventional methods could be used more effectively for the *Salmonella* isolation from foods than their single usage. Consequently, the identified *Salmonella* spp. isolates (15 isolates) were evaluated in terms of antibiotic resistance and susceptibility. The results showed that all

Salmonella spp. isolated from chicken meat samples had multi drug resistance.

Table 3. Antibiotic susceptibility of the obtained *Salmonella* spp. isolates (n:15)

Antibiotic	Susceptible	
	%	Number of <i>Salmonella</i> isolates
Ciprofloxacin (CIP)	100	15
Sulphamethoxazole/ Trimethoprim (SXT)	66.6	10
Chloramphenicol (C)	100	15
Streptomycin (S)	33.3	5
Ampicillin (AMP)	26.6	4
Gentamicin (CN)	100	15
Kanamycin (K)	6.6	1
Nalidixic Acid (NA)	66.6	10
Trimethoprim (W)	80.0	12
Tetracycline (TE)	86.6	13

n: *Salmonella* positive sample number

Table 4. The antimicrobial effects of different antibiotics on the *Salmonella* spp. isolated from the samples Antibiotics (Zone Diameter, mm)

Samples*	(CIP)	(SXT)	(C)	(S)	(AMP)
Control*	29.25±0.35 ^{Af}	31.25±0.35 ^{Ade}	30.10±0.14 ^{Bfe}	30.50±0.71 ^{Ade}	30.25±0.35 ^{Bc}
L1	24.00±0.00 ^{Dg}	25.10±0.14 ^{Df}	26.15±0.21 ^{De}	25.00±0.00 ^{Cf}	27.10±0.14 ^{Cdc}
L2	25.10±0.14 ^{Cg}	30.25±0.35 ^{Bb}	27.25±0.35 ^{Ce}	24.10±0.14 ^{Dh}	31.25±0.35 ^{Aa}
L3	0.00±0.00 ^{Gf}	0.00±0.00 ^{Gf}	0.00±0.00 ^{Hf}	0.00±0.00 ^{Ff}	0.00±0.00 ^{Df}
L7	0.00±0.00 ^G	0.00±0.00 ^{Ge}	0.00±0.00 ^{He}	0.00±0.00 ^{Fe}	0.00±0.00 ^{De}
C1	20.00±0.00 ^{Ffg}	19.50±0.71 ^{Fg}	20.00±0.00 ^{Gfg}	20.20±0.28 ^{Eefg}	20.50±0.71 ^{Cdef}
C3	0.00±0.00 ^{Gb}	0.00±0.00 ^{Gb}	0.00±0.00 ^{Hb}	0.00±0.00 ^{Fb}	0.00±0.00 ^{Db}
C6	22.00±0.00 ^{Ee}	26.25±0.35 ^{Cb}	25.35±0.49 ^{Ec}	24.25±0.35 ^{Dd}	25.25±0.35 ^{Dc}
C9	27.25±0.35 ^{Bd}	30.25±0.35 ^{Bb}	32.00±0.00 ^{Aa}	30.25±0.35 ^{Ab}	30.25±0.35 ^{Bb}
C15	20.25±0.35 ^{Ff}	24.10±0.14 ^{Ec}	24.40±0.57 ^{Fc}	27.10±0.14 ^{Ba}	27.39±0.16 ^{Ca}
B5	30.25±0.35 ^{Ae}	26.75±0.35 ^{Cd}	24.85±0.21 ^{Dg}	0.00±0.00 ^{Ff}	0.00±0.00 ^{Fe}
B6	31.75±0.35 ^{Ac}	26.20±0.28 ^{Ce}	26.00±0.00 ^{Cf}	0.00±0.00 ^{Ff}	0.00±0.00 ^{Fe}
B8	25.25±0.35 ^{Ah}	0.00±0.00 ^{Eh}	25.00±0.00 ^{Ag}	11.25±0.35 ^{Cd}	25.20±0.28 ^{Ab}
B11	30.00±0.00 ^{Afe}	28.25±0.35 ^{Bb}	26.25±0.35 ^{Cf}	0.00±0.00 ^{Gf}	0.00±0.00 ^{Ge}
B12	24.25±0.35 ^{Bi}	0.00±0.00 ^{Eh}	28.25±0.35 ^{Ad}	12.25±0.35 ^{Dc}	19.75±0.35 ^{Cc}
B15	34.25±0.35 ^{Ab}	27.25±0.35 ^{Cc}	30.25±0.35 ^{Bb}	0.00±0.00 ^{Gf}	0.00±0.00 ^{Ge}

Table 4 continuing

Samples*	(CN)	(K)	(NA)	(W)	(TE)
Control*	27.25±0.35C ^g	25.25±0.35A ^h	34.25±0.35A ^b	26.10±0.14B ^h	36.50±0.71A ^a
L1	0.00±0.00G ^h	0.00±0.00D ^h	25.20±0.28D ^f	0.00±0.00G ^h	30.25±0.35C ^a
L2	29.25±0.35A ^c	23.25±0.35B ⁱ	30.10±0.14C ^b	30.25±0.35A ^b	26.25±0.35D ^f
L3	13.25±0.35E ^b	0.00±0.00D ^f	0.00±0.00F ^f	10.25±0.35E ^c	20.25±0.35F ^a
L7	28.10±0.14B ^a	0.00±0.00D ^e	0.00±0.00F ^e	0.00±0.00G ^e	15.25±0.35G ^d
C1	20.15±0.21D ^e f ^g	22.20±0.28C ^c	24.25±0.35E ^b	20.25±0.35D ^e f ^g	25.10±0.14E ^a
C3	0.00±0.00G ^b	0.00±0.00D ^b	0.00±0.00F ^b	0.00±0.00G ^b	25.25±0.35E ^a
C6	0.00±0.00G ^f	0.00±0.00D ^f	25.25±0.35D ^c	0.00±0.00G ^f	30.00±0.00C ^a
C9	0.00±0.00G ^g	0.00±0.00D ^g	32.20±0.28B ^a	21.00±0.00C ^f	31.50±0.71B ^b
C15	0.83±0.04F ⁱ	0.00±0.00D ⁱ	24.25±0.35E ^c	7.25±0.35F ^h	25.20±0.28E ^b
B5	20.25±0.35E ^e f ^g	0.00±0.00F ^b	24.90±0.14D ^c	29.25±0.35B ^c	25.25±0.35D ^b
B6	22.75±0.35E ^c	0.00±0.00F ^b	26.25±0.35C ^b	27.75±0.35B ^d	23.75±0.35D ^d
B8	21.00±0.00B ^d e	0.00±0.00E ^b	0.00±0.00E ^f	0.00±0.00E ^g	8.50±0.71D ^g
B11	18.25±0.35F ^h	0.00±0.00G ^b	24.25±0.35D ^d	27.75±0.35B ^d	21.50±0.71E ^e
B12	20.25±0.35C ^e f ^g	0.00±0.00E ^b	0.00±0.00E ^f	24.50±0.71B ^e	0.00±0.00E ⁱ
B15	21.25±0.35F ^d	0.00±0.00G ^b	24.00±0.00D ^d	30.25±0.35B ^b	23.25±0.35E ^d

*: *Salmonella* Typhimurium. Different uppercase letters indicate significant differences ($P < 0.01$) among the antibiotics, while lowercase letters showed differences among the samples

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