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Isolation and molecular characterization of *Salmonella enterica* and *Escherichia coli* from poultry samples

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Abstract: Innumerable foodborne pathogens including *Salmonella* and *Escherichia coli* pose a serious threat to human health and food safety. As perishable foods, poultry products are considered one of the most common sources of foodborne pathogens including *Salmonella* and *E. coli* due to transmission of drug resistance, dissemination of organisms, and cross-contamination. In our study, phenotypic and genotypic characterizations of *Salmonella enterica* and *E. coli* isolated from packaged raw chicken products were carried out. Samples belonging to different commercial brands were collected in Ankara in 2015. Among 15 out of 19 *Salmonella enterica* subsp. *enterica* strains isolated from different and/or same poultry samples were found as Infantis serotype, while 4 of them were identified as Enteritidis serotype by pulsed-field gel electrophoresis (PFGE) footprints. In addition, 19 out of 40 samples gave positive results for *E. coli*. In addition, PFGE types of *Salmonella* Infantis isolates were detected as PT 08, 45, and 50. Furthermore, multilocus sequence typing types of the samples were identified as ST 32. Results of the phenotypic and genotypic antimicrobial resistance profiles of *Salmonella* Infantis and *E. coli* isolates revealed considerable resistance to nalidixic acid, tetracycline, streptomycin, sulfisoxazole and trimethoprim/sulfamethoxazole. On the other hand, 3 *E. coli* isolates showed antibiotic susceptibility. All in all, this study might enlighten some molecular features of *Salmonella* and *E. coli* isolated from chicken products in Turkey.

Key words: Antimicrobial resistance, pulsed-field gel electrophoresis, multilocus sequence typing, *Salmonella*, *E. coli*

1. Introduction

Food might act as a crucial vehicle for transmission of illnesses from animals to humans. Foodborne zoonotic pathogens, mainly *Campylobacter* spp., *Salmonella* spp., and Shiga toxin producing *E. coli,* accommodate in intestinal tract of chicken, cattle, and swine and may induce foodborne diseases (1,2). Zoonotic bacteria present in poultry pose a major risk for both poultry industry and human health by increasing antibiotic resistance and contamination. To illustrate, more than 50 *Salmonella* infections in live poultry were observed, resulting in 2630 illnesses, 387 hospitalizations, and 5 deaths in the USA from 1999 to 2014 (3). Moreover, avian pathogenic *E. coli* might lead to serious flock mortality (4). In addition, *E. coli* outbreaks occurred in France and Germany in 2011 due to verocytotoxin producing *E. coli*. A total of 3126 cases and 17 deaths related to this bacterium were reported in Germany and the European Union (EU) (5).

Salmonellosis, a nontyphoidal *Salmonella* infection, has been gradually increasing in Turkey and in other countries as a consequence of consuming poultry meat and its derivatives. Salmonellosis caused by *Salmonella enterica* subsp. *enterica* might lead to symptoms such as gastrointestinal infections and bloody diarrhea within 12 to 72 h (6). The most common *Salmonella enterica* serovars are Enteritidis, Typhimurium, and Infantis isolated from broilers, turkeys, pig meat, and human sources in Europe (7), however, in the USA, Kentucky, Enteritidis, Montevieo, Typhimurium and Infantis are frequently observed serotypes in animal products (8). According to the European Food Safety Authority and the European Centre for Disease Prevention and Control, among a total of 4786 foodborne outbreaks, *Salmonella* has been the most frequently detected foodborne pathogen, including *Salmonella* serovar Enteritidis and Infantis (9). Furthermore, the most significant increase in *Salmonella* infections was due to serotype Infantis in 2016 (10). Although Turkey occupies an important position in exporting chicken meat products, the data related to foodborne infections in Turkey is inadequate. Global food trading has expedited the emergence and spread of antibiotic resistant *Salmonella*. Hence, our study might provide useful information to trace the footprints of *Salmonella* outbreaks originating from the poultry products in Turkey.

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Multidrug-resistant strains have become more difficult to treat in recent years. The question of how antibiotic resistant bacteria acquire resistance maintains its importance. For example, resistance to ampicillin, tetracyclines, and sulfonamides in *Salmonella* was commonly determined (9). In addition, extended-spectrum beta-lactamases (ESBL) and AmpC-carbapenemase production monitored in *Salmonella* and *E.coli* (9). Moreover, antibiotic-resistant *Salmonella* Infantis has been one of the prevalent serovars in poultry products (11). Microbial subtyping has an important role in classification and characterization of foodborne pathogens such as *Salmonella* and *E. coli* (12). There are two types of typing methods consisting of phenotypic and genotypic typing methods. While serotyping, phage-typing, and antimicrobial resistance typing, which are phenotype-based typing methods, are used for *Salmonella* (12), and serotyping, biotyping, phage typing, and multilocus enzyme electrophoresis are commonly used for *E. coli* (13). On the other hand, mostly applied genotypic subtyping methods for *Salmonella* and *E. coli* are pulsed-field gel electrophoresis (PFGE), multiplelocus variable-number tandem repeat Analysis (MLVA), ribotyping, plasmid profile analysis, and multilocus sequence typing (MLST) (12,13). PFGE associated with gold standard method uses restriction enzymes specified by uncommon recognition sites such as *Xba*I, *Bln*I, *Spe*I, *Avr*II, resulting in large DNA fragments varying from 20 kb to 800 kb (12,14,15). Moreover, the sequences of multiple housekeeping genes, which are highly conserved, are analyzed in MLST method (16). For *Salmonella* MLST scheme, the most commonly used housekeeping genes are *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA* (17), while for *E. coli* MLST scheme, *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA* are some examples for housekeeping genes (18). All in all, our study aimed to investigate the genetic diversity of *Salmonella* and *E.coli* isolated from raw chicken products by PFGE using restriction enzyme *Xba*I, and MLST. Furthermore, the resistance of *Salmonella* and *E.coli* isolates to antimicrobials was tested both phenotypically and genotypically. Additionally, biofilm forming abilities of *Salmonella* isolates were analyzed.

2. Materials and methods

2.1. Poultry samples

Forty packaged raw chicken products belonging to different commercial brands from the local markets in Ankara were collected in 2015 (Table 1). In order to isolate *Salmonella* and *E.coli* strains, raw chicken materials were categorized into seven different parts, namely chicken breast, wing, heart, gizzard, rib, chop, and drumstick (Table 1). All isolates were freezed in 15% glycerol solution at –80 °C at Middle East Technical University, Department of Food Engineering.

2.2. Isolation of *Salmonella*

For *Salmonella* isolation, the international standard ISO6579:2002 was used. After 25 g of sample from each chicken product were incubated in buffered peptone water at 37 °C overnight, 1 mL of each broth sample was transferred to 10 mL Rappaport Vassiliadis soya peptone broth (RVS broth, CM0866 Oxoid) and incubated at 44 °C for 24 h. Ten microliter of RVS broth from each sample was spread on the Brilliant Green Agar (BGA, CM0263 Oxoid) plate and Xylose-Lysine-Desoxycholate Agar (XLD Agar, CM0469 Oxoid) plate and incubated at 37 °C for 24 h. At least three red colonies with black centers on XLD agar, and pink colored colonies on BGA agar were selected as *Salmonella*. To confirm the suspected *Salmonella* colonies, *inv*A gene was screened by PCR (19). For one sample, 15.5 µL ddH$_2$O, 5 µL 5X Go Taq Flexi Buffer, 1.5 µL MgCI$_2$, 0.5 µL dNTPs, 1.0 µL of each primer (Forward primer: GAATCCTCAGTTTTTCAACGTTTC; Reverse primer: TAGCCGTAACAACCAATACAAATG (19)) and 0.125 µL GoTaq DNA polymerase and 0.375 µL DNA template were prepared under the following conditions: initial denaturation at 94 °C for 10 min, denaturation at 94 °C for 60 s, annealing at 60 °C for 60 s, extension at 72 °C for 60 s with 35 cycles, and the last extension for 72 °C for 7 min. Three of the confirmed *Salmonella* colonies from each sample were frozen in glycerol stock and stored in our foodborne pathogen database with a Middle East Technical University Identification (METU ID) as "MET", resulting in a total of 57 *Salmonella* strains which are shown in Table 1.

2.3. *E. coli* isolates

E. coli strains (Table 1) were isolated from 40 packaged raw chicken products as per the international standard ISO16654:2001 in a parallel way with the isolation of *Salmonella* strains. Yellow colonies on XLD agar were chosen as *E. coli*. These selected colonies were incubated on Brain Heart Infusion (BHI, CM1136 Oxoid) agar plate at 37 °C for 24 h. To confirm the selected colonies as *E. coli*, *rpoB* gene was screened by PCR. For one sample, 16.25 µL ddH$_2$O, 2.5 µL 10X Buffer solution, 1.5 µL MgCI$_2$, 0.5 µL dNTPs, 1.0 µL of each primer (Forward: GTATGTCCAATCGAAACCCCT; Reverse: GGTAGTGAATTTCGTCAGTTACA (20)), 0.25 µL 2.5 U Taq DNA Polymerase, and 2 µL DNA template were prepared under the following conditions: initial denaturation at 94 °C for 10 min, denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 seconds, with 35 cycles and the last extension for 72 °C for 5 min. Confirmed isolates were frozen as mentioned above.

Table 1. Bacterial strains isolated from packaged raw chicken products.*

Isolate code	Isolate source	Brand**	Bacterial strain	Isolation date	Location
MET S1-750	**Chicken wing**	**Z**	***Salmonella* Infantis**	**26.01.2015**	**Ankara**
MET S1-753	**Chicken heart**	**V**	***Salmonella* Infantis**	**26.01.2015**	**Ankara**
MET S1-756	Chicken drumstick	V	*Salmonella* Enteritidis	26.01.2015	Ankara
MET S1-759	**Chicken breast**	**Y**	***Salmonella* Infantis**	**28.01.2015**	**Ankara**
MET S1-762	Chicken gizzard	Z	*Salmonella* Enteritidis	28.01.2015	Ankara
MET S1-765	**Chicken breast**	**Z**	***Salmonella* Infantis**	**28.01.2015**	**Ankara**
MET S1-768	Chicken wing	W	*Salmonella* Enteritidis	01.02.2015	Ankara
MET S1-771	Chicken breast	W	*Salmonella* Enteritidis	01.02.2015	Ankara
MET S1-774	**Chicken rib**	**W**	***Salmonella* Infantis**	**01.02.2015**	**Ankara**
MET S1-777	**Chicken drumstick**	**P**	***Salmonella* Infantis**	**01.02.2015**	**Ankara**
MET S1-780	**Chicken wing**	**P**	***Salmonella* Infantis**	**01.02.2015**	**Ankara**
MET S1-782	**Chicken wing**	**P**	***Salmonella* Infantis**	**01.02.2015**	**Ankara**
MET S1-785	**Chicken drumstick**	**P**	***Salmonella* Infantis**	**01.02.2015**	**Ankara**
MET S1-788	**Chicken breast**	**M**	***Salmonella* Infantis**	**01.02.2015**	**Ankara**
MET S1-792	**Chicken heart**	**Q**	***Salmonella* Infantis**	**02.02.2015**	**Ankara**
MET S1-795	**Chicken breast**	**Q**	***Salmonella* Infantis**	**02.02.2015**	**Ankara**
MET S1-798	**Chicken heart**	**Q**	***Salmonella* Infantis**	**26.02.2015**	**Ankara**
MET S1-801	**Chicken breast**	**Q**	***Salmonella* Infantis**	**26.02.2015**	**Ankara**
MET S1-804	**Chicken wing**	**Q**	***Salmonella* Infantis**	**26.02.2015**	**Ankara**
MET A1-001	**Chicken breast**	**W**	***Escherichia coli***	**01.02.2015**	**Ankara**
MET A1-002	**Chicken drumstick**	**P**	***Escherichia coli***	**01.02.2015**	**Ankara**
MET A1-003	**Chicken wing**	**Q**	***Escherichia coli***	**02.02.2015**	**Ankara**
MET A1-004	**Chicken drumstick**	**P**	***Escherichia coli***	**01.02.2015**	**Ankara**
MET A1-005	**Chicken drumstick**	**J**	***Escherichia coli***	**27.01.2015**	**Ankara**
MET A1-007	**Chicken wing**	**W**	***Escherichia coli***	**01.02.2015**	**Ankara**
MET A1-008	**Chicken breast**	**Q**	***Escherichia coli***	**02.02.2015**	**Ankara**
MET A1-009	**Chicken chop**	**Q**	***Escherichia coli***	**02.02.2015**	**Ankara**
MET A1-010	**Chicken wing**	**P**	***Escherichia coli***	**01.02.2015**	**Ankara**
MET A1-011	**Chicken wing**	**Q**	***Escherichia coli***	**02.02.2015**	**Ankara**
MET A1-012	**Chicken wing**	**Q**	***Escherichia coli***	**02.02.2015**	**Ankara**
MET A1-014	**Chicken wing**	**Z**	***Escherichia coli***	**27.01.2015**	**Ankara**
MET A1-015	**Chicken drumstick**	**Q**	***Escherichia coli***	**02.02.2015**	**Ankara**
MET A1-016	**Chicken wing**	**P**	***Escherichia coli***	**01.02.2015**	**Ankara**
MET A1-017	**Chicken chop**	**W**	***Escherichia coli***	**01.02.2015**	**Ankara**
MET A1-018	**Chicken drumstick**	**P**	***Escherichia coli***	**01.02.2015**	**Ankara**
MET A1-019	**Chicken wing**	**Q**	***Escherichia coli***	**02.02.2015**	**Ankara**
MET A1-020	**Chicken drumstick**	**W**	***Escherichia coli***	**01.02.2015**	**Ankara**
MET A1-021	**Chicken drumstick**	**P**	***Escherichia coli***	**01.02.2015**	**Ankara**

* Bold *Salmonella* Infantis and *Escherichia coli* isolates were used in this study.
** Commercial brands' names were substituted by the letters.

2.4. Pathogenicity of *E. coli* isolates

E. coli is composed of various groups of bacteria. Pathogenic *E. coli* strains are divided into several pathotypes. In our study, the genes (Table 2) related with Shigatoxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), and Diffusely Adherent *E. coli* (DAEC) were screened by PCR (21–28). Confirmed isolates were frozen as mentioned above.

2.5. PFGE typing of bacterial strains

PFGE analysis, known as a gold standard for both *Salmonella* and *E. coli,* was carried out according to the PulseNet protocol (29). Isolates streaked on BHI agar were incubated at 37 °C for 14–18 h. Using sterile cotton swabs, the cultures were transferred to 4 mL of cell suspension buffer. After the absorbance of the mixture of cell and the buffer was fixed to 1.3–1.4 at 610 nm by the spectrophotometer, 20 µL of proteinase-K was added to 400 µL of mixture for each sample. Plugs were formed including Seakem Agarose with 1% SDS and cell-buffer mixtures. They were added to 5 mL of cell lysis buffer and washed with sterile deionized water two times and then washed with Tris-EDTA (TE) buffer four times at 50 °C. DNA restriction was done by *Xba*I at 37 °C for 4 h. Restricted DNACHEF-DR III was run with the reference strain *Salmonella* Braenderup H9812 by BioRad under specified PFGE electrophoresis conditions: 6.0 V/cm, 19 h, 120°, 2.16–63.8 s, and 70 (0.75 dm^3/min). DNA bands were screened using Quantity One software and Molecular Imager-Gel Doc-XR System Universal Hood II.

Table 2. Primers used in determining pathogenicity of *E. coli* isolates.

	Gene	Primer Sequences	Annealing temperature (°C)	Product size (bp)	Reference
STEC genes	*fliC*	F: AGCTGCAACGGTAAGTGATTT R: GGCAGCAAGCGGGTTGGTC	65.0	949	(21)
	stx1	F: TGTCGCATAGTGGAACCTCA R: TGCGCACTGAGAAGAAGAGA	65.0	655	(22)
	stx2	F: CCATGACAACGGACAGCAGTT R: TGTCGCCAGTTATCTGACATTC	65.0	477	(23)
	eae	F: CATTATGGAACGGCAGAGGT R: ACGGATATCGAAGCCATTTG	65.0	375	(22)
	rfbE	F: CAGGTGAAGGTGGAATGGTTGTC R: TTAGAATTGAGACCATCCAATAAG	65.0	296	(24)
	hlyA	F: GCGAGCTAAGCAGCTTGAAT R: CTGGAGGCTGCACTAACTCC	65.0	199	(22)
EPEC genes	*bfpA*	F: AATGGTGCTTGCGCTTGCTGC R: GCCGCTTTATCCAACCTGGTA	59.0	326	(25)
	eaf	F: CAGGGTAAAAGAAAGATGATAA R: TATGGGGACGTATTATCA	59.0	397	(26)
ETEC genes	*st*	F: ATTTTTMTTTCTGTATTRTCTT R: CACCCGGTACARGCAGGATT	50.0	190	(27)
	lt	F: GGCGACAGATTATACCGTGC R: CGGTCTCTATATTCCCTGTT	50.0	450	(27)
EIEC gene	*ipaH*	F: GTTCCTTGACCGCCTTTCCGATACCGTC R: GCCGGTCAGCCACCCTCTGAGAGTAC	60.0	619	(28)
EAEC gene	*aggR*	F: CGAAAAAGAGATTATAAAAATTAAC R: GCTTCCTTCTTTTGTGTAT	60.0	100	(28)
DAEC gene	*daaD*	F: TGAACGGGAGTATAAGGAAGATG R: GTCCGCCATCACATCAAAA	60.0	444	(28)

*F: forward primer, R: reverse primer.

The pictures of PFGE gels were analyzed by BioNumerics version 7.6 (Applied-Maths, Kortrijk, Belgium).

2.6. MLST of *Salmonella* isolates

MLST, characterizing the isolates by considering the internal fragments of seven house-keeping genes (Table 3), including *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*, was carried out (30). In addition, nucleotide sequences were analyzed by DNASTAR Lasergene software. After *Salmonella* taken from –80 °C was streaked on BHI agar at 37 °C overnight, a colony was incubated in BHI broth at 37 °C overnight for each sample. Cells were lysed by microwave. Lysate of *Salmonella* DNA were used for PCR mixture by applying 71.5 µL ddH_2O, 10.0 µL 10X PCR buffer solution, 6.0 µL $MgCI_2$, 2.0 µL dNTPs, 4.0 µL of each primer given in Table 3, 0.5 µL Taq DNA polymerase and 2.0 µL DNA template for each 7 genes mentioned above under the following conditions: initial denaturation at 94 °C for 10 min, denaturation at 94 °C for 60 s, annealing at 60 °C for 60 s, extension at 72 °C for 60 s with 35 cycles, and the last extension for 72 °C for 7 min. *Salmonella* databank of University College Cork was also used in order to determine allelic profile or sequence type.

2.7. Biofilm detection

Biofilm formations of pathogenic bacteria on abiotic surfaces such as stainless steel, aluminum, polystyrene and plastic, and food contact surfaces lead to serious threat for the public human health and food industry (31,32). *Salmonella* is one of the most prevalent microorganisms forming biofilm in chicken flocks (33). Hence, a well-known method was implemented to observe the biofilm forming capabilities of *Salmonella* strains isolated from packaged raw chicken products (34). *Salmonella* Salford was used as a positive control in this procedure. Strains were incubated at 37 °C for 16 h in 10 mL Brain Heart Infusion broth (BHI, CM1136, Oxoid). 230 µL Tryptone Soya Broth (TSB, CM0129, Oxoid) and 20 µL of incubated *Salmonella* culture were transferred to sterile 96-well plates with three replicates. After the incubation at 37 °C for 24 h, they were washed with 0.85% NaCI solution three times. Fixation process was done using 96% MeOH. Then, 250 µL crystal violet was added to 96-well plates and biofilm formations were observed.

2.8. Antimicrobial susceptibility analysis

19 different antimicrobial agents, amikacin (30 µg) , gentamicin (10 µg), kanamycin (30 µg), streptomycin (10 µg), ampicillin (10 µg), ceftiofur (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), cephalothin (30 µg), amoxicillin-clavulanic acid (20µg/10 µg), ertapenem (10 µg), imipenem (10 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25µg/23.75 µg), and sulfisoxazole (300 µg), were applied using disc diffusion method. Cell cultures were incubated in Mueller-Hinton (CM0405, Oxoid) broth at 37 °C for 2–8 h. After the incubation, microbial density was adjusted to 1–2 × 10^8 CFU/mL with 0.5 McFarland standard. Cultures on Mueller-Hinton agar

Table 3. Primers used in amplifying seven house-keeping genes for MLST analysis of *Salmonella* isolates.

Gene	Primer sequences	Product size (bp)	Reference
aroC	F: GGCACCAGTATTGGCCTGCT R: CATATGCGCCACAATGTGTTG	826	(30)
thrA	F: GTCACGGTGATCGATCCGGT R: CACGATATTGATATTAGCCCG	852	(30)
purE	F: ATGTCTTCCCGCAATAATCC R: TCATAGCGTCCCCCGCGGATC	510	(30)
sucA	F: AGCACCGAAGAGAAACGCTG R: GGTTGTTGATAACGATACGTAC	643	(30)
hisD	F: GAAACGTTCCATTCCGCGCAGAC R: CTGAACGGTCATCCGTTTCTG	894	(30)
hemD	F: ATGAGTATTCTGATCACCCG R: ATCAGCGACCTTAATATCTTGCCA	666	(30)
dnaN	F: ATGAAATTTACCGTTGAACGTGA R: AATTTCTCATTCGAGAGGATTGC	833	(30)

*F: forward primer, R: reverse primer.

with discs (6 mm) were incubated at 37 °C for 16–18 h. *E. coli* ATCC25922 was used as a control isolate for diffusion tests. Antimicrobial resistance results were adjusted according to Clinical Laboratory Standards Institute (35,36).

2.9. Antimicrobial gene screening

In this stage, bla_{TEM-1}, $bla_{PSE-}1$, $blaCMY_{-2}$, *ampC*, *cat1*, *cat2*, *flo*, *cmlA*, *aadA1*, *aadA2*, *strA*, *strB*, *aacC2*, *aphA1-Iab*, *dhfrI*, *dhfrXII*, *sulI*, *sulII*, *tetA*, *tetB*, *and tetG* genes (Table 4) encoding antimicrobial resistance were analyzed (37–41). Purified *Salmonella* and *E. coli* DNA were used by adding 71.50 µL ddH_2O, 10.0 µL 10X PCR buffer, 6.0 µL $MgCI_2$, 2.0 µL dNTPs, 4.0 µL of each primer, 0.5 µL Taq DNA Polymerase, and 2.0 µL DNA template with the certain annealing temperature of the primers listed in Table 4. Five microliter of PCR product with DNA marker was run on 1.5% agarose gel at 110 V for 1 h.

3. Results and discussion

3.1. *Salmonella* Infantis and *E.coli* isolates

Nineteen *Salmonella enterica* subsp. *enterica* isolates were collected from different raw chicken products (Table 1). Fifteen out of 19 isolates were determined as *Salmonella* Infantis and these 15 *Salmonella* Infantis isolates were used in this study (Table 1). On the other hand, 19 *E.coli* isolated from different parts of raw chicken products were confirmed by *rpoB* gene (Table 1).

3.2. Pathogenicity of *E. coli* isolates

E. coli is the most predominant commensal bacteria found in the gastrointestinal tracts of warm-blooded animals and humans (42). On the other hand, as pathogenic bacteria it leads to serious bacterial infections such as diarrhea, enteritis, septicemia, and urinary tract infection at the same time (43). Hence, pathogenic *E. coli* strains including certain virulence factors are categorized considering O (somatic) and H (flagellar) antigens (42). In other words, pathogenic *E. coli* influencing the human intestines are classified into six main groups containing STEC, EPEC, ETEC, EIEC, EAEC, and DAEC (44). For example, confirmation of STEC strains is done by conventional PCR marking *stx*, *eae*, and genetic codes for the O- and H-antigens (22–25), while EPEC adherence factor and bundle-forming pilus are taken into consideration for the determination of EPEC strains (26,27). In addition, genes expressing heat-labile (LT) and heat-stable (ST) enterotoxins, genetic code for the invasion related pathogen antigen, virulence gene encoding Fimbria AAF/I, and F1845 fimbrial adhesion genes play crucial roles in identifying ETEC, EIEC, EAEC, and DAEC strains, respectively (27,28). Pathogenicity genes (Table 2) including *fliC* (flagellar antigen), *stx1* (Shiga toxin 1), *stx2* (Shiga toxin 2), *eae* (intimin), *rfbE* (O157 antigen), *hlyA* (hemolysin), *bfpA* (BfpA protein), *eaf* (adherence factor), *st* (ST enterotoxin), *lt* (LT enterotoxin) , *ipaH* (invasion plasmid antigen H), *aggR* (adherence factor), and *daaD* (adherence factor) could not be determined in *E. coli* isolates, used in this study. This result revealed that *E. coli* isolates, collected in this study, are considered as commensal bacteria. Moreover, occurrence of both pathogenic and commensal bacteria in raw chicken products was confirmed.

3.3. Biofilm-forming capabilities of *Salmonella* isolates

Salmonella isolates were not found to be as biofilm-forming foodborne pathogens on abiotic surfaces. In terms of forming biofilm on different surfaces such as abiotic and biotic, bacterial strains might demonstrate biofilm-forming variations. Moreover, poultry-houses, water supply systems and climatic conditions such as temperature and humidity play crucial roles in biofilm formation. For further studies, abilities of biofilm formation on food surfaces such as chicken meat might be analyzed.

3.4. PFGE Typing of *Salmonella* and *E.coli* isolates

Fifteen out of 19 *Salmonella* isolated from raw chicken wing, heart, breast, rib and drumstick were verified as *Salmonella* Infantis while 4 isolates were confirmed as Enteritidis.

Nineteen *Salmonella* isolates were typed genotypically by PFGE (Figure 1). Fifteen of them were confirmed as *Salmonella* Infantis comparing the footprints of Infantis isolates known before. Likewise, since 4 isolates shared the same PFGE patterns with *Salmonella* Enteritidis in our database, these 4 isolates were assigned as *Salmonella* Enteritidis (Figure 2). Dendograms of Infantis and Enteritidis isolates created by BioNumerics analyses were shown in Figures 1 and 2, respectively.

A total of 15 *Salmonella* Infantis isolates, used in this study, only represent three PFGE patterns (PT) (i.e. PT 08, PT 45, and PT 50). Slight variations in PFGE patterns were observed. Moreover, while the majority of Infantis isolates (N=13) represent PT 08, only two isolates were differentiated into two different PFGE patterns, PT 45 and PT 50; PT 45 was observed in MET S1-753, and PT 50 was observed in MET S1-777. In addition, isolates verified as PT 08 showed multidrug resistance.

In contrast to our study, *Salmonella enterica* serovar Enteritidis has been increasing in laying hens and broilers in some European Union countries such as Greece, Poland, Spain, and Romania (9). On the other hand, *Salmonella* Infantis isolated from human and food sources has been one of the most frequently found serovar in Brazil for more than 25 years (45). In addition, Infantis still keeps posing a serious problem to public health all around the world including European countries, Morocco, Japan, and the USA (10, 46–48).

Table 4. Primer sequences used in genotypic antimicrobial susceptibility.

Gene	Antibiotic resistance	Primer sequence	Binding temperature (ºC)	Reference
bla $_{TEM-1}$	Class A β-lactam	F: CAGCGGTAAGATCCTTGAGA R: ACTCCCCGTCGTGTAGATAA	53.9	(37)
bla $_{PS1E-1}$	Class A β-lactam	F: TGCTTCGCAACTATGACTAC R: AGCCTGTGTTTGAGCTAGAT	52.4	(37)
bla $_{CMY-2}$	Ceftiofur, Ceftriaxone	F: TGGCCGTTGCCGTTATCTAC R: CCCGTTTTATGCACCCATGA	60.8	(37)
ampC	β-lactams	F: AACACACTGATTGCGTCTGAC R: CTGGGCCTCATCGTCAGTTA	60.0	(38)
cat1	Chloramphenicol	F: CTTGTCGCCTTGCGTATAAT R: ATCCCAATGGCATCGTAAAG	Touchdown 55.0-45.0	(37)
cat2	Chloramphenicol	F: AACGGCATGATGAACCTGAA R: ATCCCAATGGCATCGTAAAG	60.0	(37)
flo	Chloramphenicol	F: CTGAGGGTGTCGTCATCTAC R: GCTCCGACAATGCTGACTAT	54.4	(37)
cmlA	Chloramphenicol	F: CGCCACGGTGTTGTTGTTAT R: GCGACCTGCGTAAATGTCAC	58.5	(37)
aadA1	Streptomycin	F: TATCAGAGGTAGTTGGCGTCAT R: GTTCCATAGCGTTAAGGTTTCATT	53.6	(39)
aadA2	Streptomycin	F: TGTTGGTTACTGTGGCCGTA R: GATCTCGCCTTTCACAAAGC	57.3	(39)
strA	Streptomycin	F: CTTGGTGATAACGGCAATTC R: CCAATCGCAGATAGAAGGC	51.8	(40)
strB	Streptomycin	F: ATCGTCAAGGGATTGAAACC R: GGATCGTAGAACATATTGGC	57.0	(40)
aacC2	Gentamicin, Kanamycin	F: GGCAATAACGGAGGCAATTCGA R: CTCGATGGCGACCGAGCTTCA	57.9	(37)
aphA1-Iab	Kanamycin	F: AAACGTCTTGCTCGAGGC R: CAAACCGTTATTCATTCGTGA	54.0	(41)
dhfrI	Trimethoprim	F: CGGTCGTAACACGTTCAAGT R: CTGGGGATTTCAGGAAAGTA	51.7	(37)
dhfrXII	Trimethoprim	F: AAATTCCGGGTGAGCAGAAG R: CCCGTTGACGGAATGGTTAG	57.9	(37)
sul1	Sulfonamide	F: TCACCGAGGACTCCTTCTTC R: CAGTCCGCCTCAGCAATATC	55.6	(37)
sul2	Sulfonamide	F:CCTGTTTCGTCCGACACAGA R: GAAGCGCAGCCGCAATTCAT	56.0	(37)
tetA	Tetracycline	F: GCGCCTTTCCTTTGGGTTCT R: CCACCCGTTCCACGTTGTTA	57.7	(37)
tetB	Tetracycline	F: CCCAGTGCTGTTGTTGTCAT R: CCACCACCAGCCAATAAAAT	58.4	(37)
tetG	Tetracycline	F: AGCAGGTCGCTGGACACTAT R: CGCGGTGTTCCACTGAAAAC	60.0	(37)

*F: forward primer, R: reverse primer.

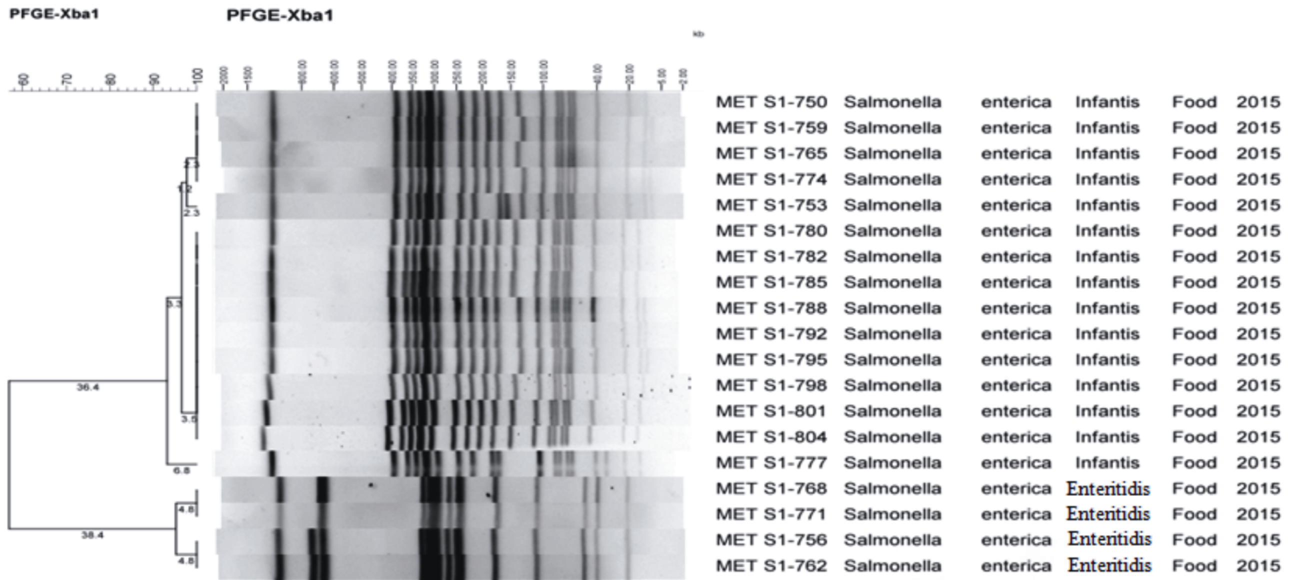

Figure 1. PFGE footprints of 19 *Salmonella* isolates.

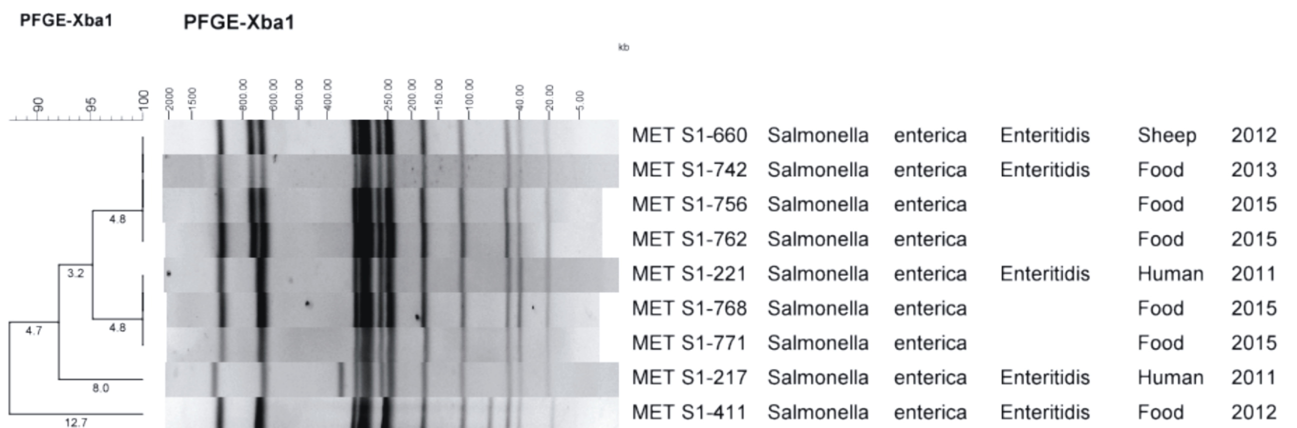

Figure 2. Dendogram of PFGE Types of 4 *Salmonella* Enteritidis isolates. *1: MET A1-001, 2: MET A1-002, 3: MET A1-003, 4: MET A1-004, 5: MET A1-005, 7: MET A1-007, 8: MET A1-008, SB: *Salmonella* Braenderup H9812.

In another research, four different PFGE patterns of *Salmonella* Infantis were commonly found in human and food sources and broilers in Germany (49). Moreover, two PFGE patterns were found in *Salmonella* Infantis predominantly in Hungary (50). Hereby, it might be concluded that variances in PFGE types change with regard to the geographical regions. And distinct PFGE footprints of *Salmonella* Infantis might be derived from high conjugative transfer rates of mobile genetic elements such as integrons and plasmids.

PFGE analysis of 19 *E. coli* isolates was carried out according to PulseNet protocol (29). As can be seen in Figures 3 and 4, PFGE footprints were observed distinct from each other. And *E. coli* isolates were also certified with different footprints by PFGE dendogram in Figure 5.

3.5. MLST of *Salmonella* isolates

With the PFGE pattern results of 15 *Salmonella* Infantis isolates, MLST types of them were identified as ST 32. Over and above that, Infantis isolated in Brazil and Morocco has showed the same allelic type, ST 32 (45,51).

3.6. Phenotypic and genotypic antimicrobial resistance analysis of *Salmonella* and *E. coli* isolates

The phenotypic analysis revealed that each *Salmonella* Infantis isolate had resistance to at least one antibiotic; all of them were resistant to nalidixic acid. Except for MET S1-753, all of the *Salmonella* Infantis isolates were accepted as multidrug-resistant foodborne pathogens due to resistance to at least two antimicrobial agents. As can be seen in Table 5, SfSxtNT (Sulfisoxazole-Trimethoprim/sulfamethoxazole-Nalidixic acid- Tetracycline)

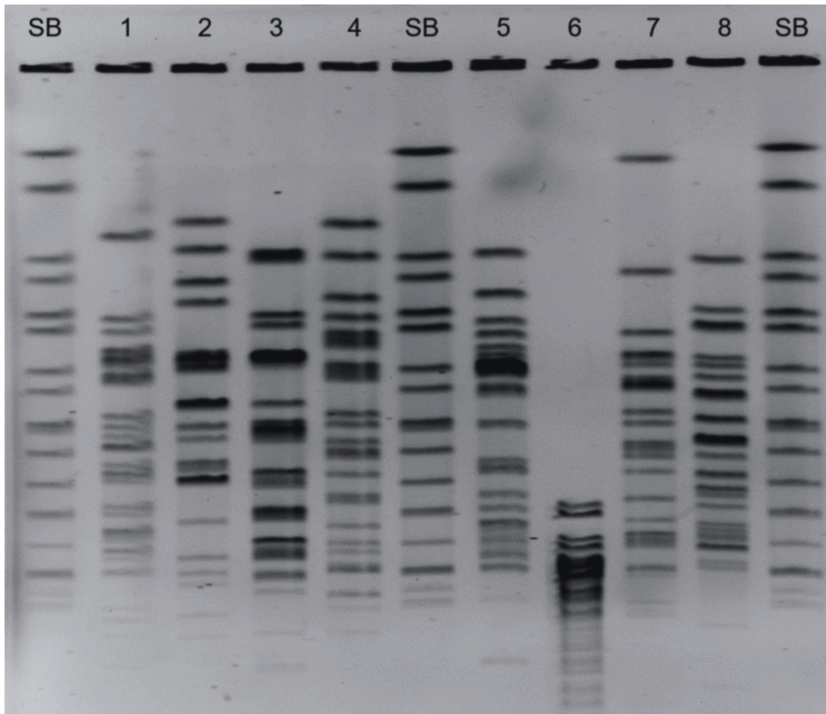

Figure 3. PFGE patterns of seven *E.coli* isolates. *009: MET A1-009, 010: MET A1-010, 011: MET A1-011, 012: MET A1-012, 014: MET A1-014, 015: MET A1-015, 016: MET A1-016, 017: MET A1-017,018: MET A1-018, 019: MET A1-019, 020: MET A1-020, 021: MET A1-021, SB: *Salmonella* Braenderup H9812.

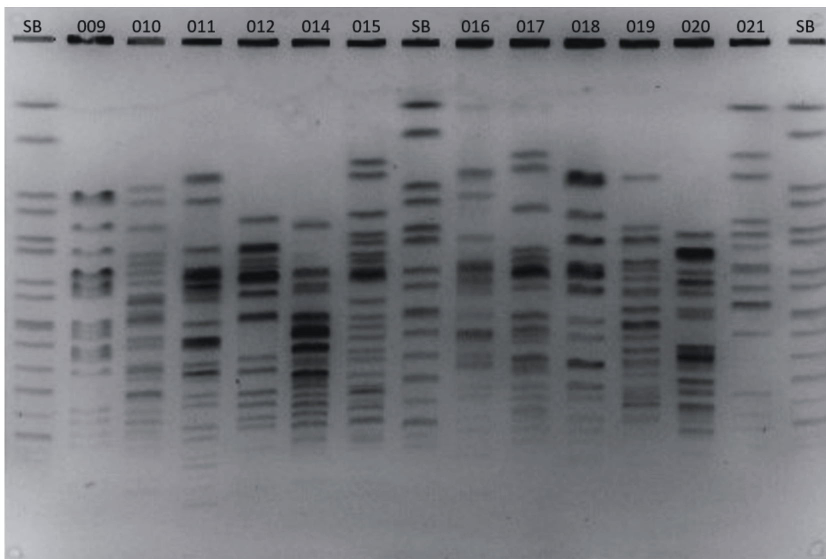

Figure 4. PFGE patterns of twelve *E. coli* isolates.

phenotypic antimicrobial resistance profile was the most observed one in Infantis isolates. To conclude, *Salmonella* Infantis isolates showed antibiotic resistance significantly to nalidixic acid (100%), tetracycline (93%), sulfisoxazole (93%), trimethoprim/sulfamethoxazole (93%), streptomycin (53%), kanamycin (47%), chloramphenicol (33%), and ciprofloxacin (13%). On the other hand, they were susceptible to amikacin, gentamicin, ampicillin, ceftiofur, cephalothin, amoxicillin-clavulanic acid, ertapenem, imipenem, ceftriaxone, and cefoxitin. Another

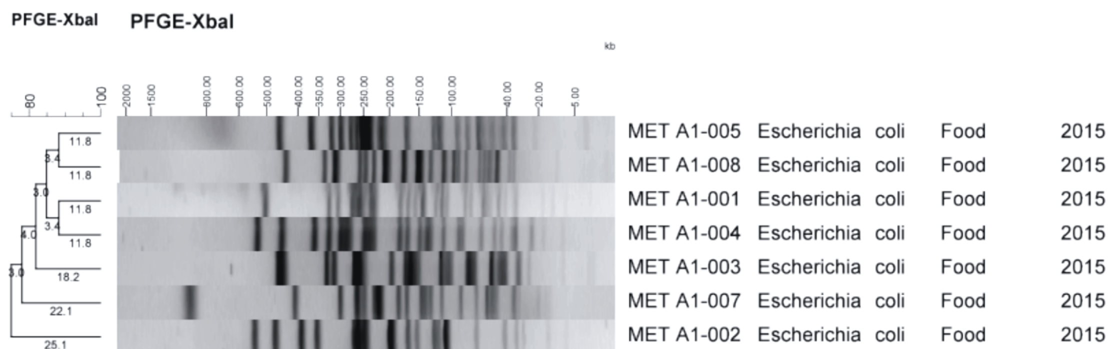

Figure 5. Dendogram of PFGE types of seven *E. coli* isolates.

research stated that multidrug-resistant *Salmonella* Infantis detected in Hungarian broilers and humans has the same phenotypic antimicrobial resistance profile including nalidixic acid, sulphonamide, streptomycin, and tetracycline as the recent Infantis strains dominating in Poland, Austria, and Hungary (47). Likewise, *Salmonella* Infantis isolates from broilers were found mostly resistant to streptomycin and sulfamethoxazole, trimethoprim as well in Japan (48). In addition, *Salmonella* Infantis isolated from broiler chickens indicated multidrug resistance via large conjugative plasmid in Hungary (50). Furthermore, *Salmonella* Infantis showed resistance to third generation cephalosporins in Belgium, due to plasmid acquisition (52). *Salmonella* Infantis isolated from clinical sources also showed antibiotic resistance in China (53). In some patients infected with *Salmonella* antibiotic treatment might be required; fluoroquinolones and third-generation cephalosporins are used for adults and children, respectively. Thus, resistance to these antibiotics might conduce to treatment failure. In contrast to the phenotypic antibiotic resistance profiles of *Salmonella* Infantis isolates used in this study, their genotypic antibiotic resistance profiles consisting of *tetA* (100%), *aadA1* (93%), *sul1* (86%), *aphA1-IAB* (66%), *strA* (20%), bla_{TEM-1} (13%), and *cmlA* (6%) were identified. However, bla_{PS1E-1}, bla_{CMY-2}, *ampC, cat1, cat2, flo, aadA2, strB, aacC2, dhfrI, dhfrXII, sul2, tetB*, and *tetG* were not detected in Infantis isolates. In China, *tetA, tetB, tetC, tetG, sul1, sul2, sul3, floR*, bla_{TEM}, and bla_{CTX-M} were commonly determined in *Salmonella enterica* serovar Enteritidis and Typhimurium isolated from broiler chickens (54). Moreover, the predominance of AmpC β-lactamase CMY-2 producing *Salmonella* isolated from chicken meat products in Japan have increased between 2005 and 2011 (55). In other studies, bla_{TEM-1}, *strA, strB, sul2, tetB, catA1, aphA-1*, and class 1 integron including *folA, catB3, aadA4*, and *sul1* gene cassettes were found in *Salmonella* Infantis isolated from human, animals and the environment in Italy (56), and *tetA, aadA1a*, and *aphA1-IAB* were ascertained in *Salmonella* Infantis isolates from poultry in Japan (48). In contradistinction to our study, strong biofilm-producing *Salmonella* Infantis strains on abiotic surface including polystyrene harbored *floR, cmlA, tetA, tetB, tetG, temB*, bla_{PS1E-1}, *sul1, sul2, qnrA, qnrS, strA*, and *aadA* antibiotic resistance genes in Malaysia (57). In summary, a lot of studies reveal highly antibiotic resistance of *Salmonella* Infantis from poultry to ampicillin, nalidixic acid, streptomycin, sulphonamide and tetracycline (58). In addition to this, bla_{TEM-1} conferring resistance to extended-spectrum beta-lactamase was confirmed in two Infantis isolates of this study (MET S1-759 and MET S1-765). Nontyphoidal *Salmonella* might acquire resistance to extended-spectrum beta-lactam antibiotics because of plasmid-mediated AmpC-type beta lactamases (59).

E. coli is part of the intestinal tract of chickens, and acts as a commensal bacterium unless any deterioration in the gut microbiota happens (60). Otherwise, *E. coli* might overgrow and lead to extraintestinal infections. Enteric bacteria frequently demonstrate resistance to a broad array of antibiotics such as ampicillin and tetracycline by means of antimicrobials extensively used in poultry production (61). In this study, 3 out of 19 *E. coli* isolates including MET A1-014, MET A1-017, META1-018 were found as susceptible to antibiotic agents. On the other hand, in their genotypic profiles, antibiotic resistance genes including *aadA2, strB* (in MET A1-014), *aadA1*, bla_{TEM-1} (in MET A1-017), *aphA1-IAB*, bla_{TEM-1}, bla_{CMY-2} (in MET A1-018) were found respectively. The rest of isolates except for MET A1-009 having cephalotin resistance demonstrated resistance to more than two antimicrobials in phenotypic level (Table 5). Antibiotic resistant commensal *E. coli* isolates showed resistance to ciprofloxacin (69%), nalidixic acid (69%), tetracycline (63%), sulfisoxazole (63%), ampicillin (63%), streptomycin (50%), cephalotin (44%), trimethoprim/sulfamethoxazole (44%), chloramphenicol (38%), kanamycin (31%), gentamicin (25%), cefoxitin (13%), amoxicillin-clavulanic acid (13%), ceftriaxone (6%), ceftiofur (6%), and ertapenem (6%). They were found susceptible to amikacin, and imipenem. In another recent study, *E. coli* isolated from food-producing animals of two poultry farms in Brazil showed high resistance to

Table 5. Phenotypic and genotypic antimicrobial resistance profiles of *Salmonella* Infantis and *E.coli* isolates.

Isolate code	Phenotypic antimicrobial resistance profile	Genotypic antimicrobial resistance profile
***Salmonella* Infantis isolates**		
MET S1-750	SfSxtKNT	*aadA1, aphA1-IAB, sul1, tetA*
MET S1-753	N	*aadA1, aphA1-IAB, tetA*
MET S1-759	SfSxtNT	bla_{TEM-1}, *aadA1, aphA1-IAB, sul1, tetA*
MET S1-765	SfSxtKNT	*aadA1, aphA1-IAB,* bla_{TEM-1}, *cmlA, sul1, tetA*
MET S1-774	SfSxtKSNT	*aphA1-IAB, sul1, tetA*
MET S1-777	SfSxtSCipNT	*aadA1, sul1, tetA*
MET S1-780	SfSxtKNT	*aadA1, aphA1-IAB, sul1, tetA*
MET S1-782	SfSxtKSNT	*aadA1, aphA1- IAB, strA, sul1, tetA*
MET S1-785	SfSxtCSNT	*aadA1, sul1, tetA*
MET S1-788	SfSxtCSCipNT	*aadA1, strA, sul1, tetA*
MET S1-792	SfSxtSNT	*aadA1, aphA1-IAB, sul1, tetA*
MET S1-795	SfSxtNT	*aadA1, strA, sul1, tetA*
MET S1-798	SfSxtCSNT	*aadA1, tetA*
MET S1-801	SfSxtCKSNT	*aadA1, aphA1-IAB, sul1, tetA*
MET S1-804	SfSxtCKNT	*aadA1, aphA1-IAB, sul1, tetA*
***E. coli* Isolates**		
MET A1-001	CroEftAmpAmcFoxKf	*ampC,* bla_{TEM-1}, bla_{CMY-}*2,*
MET A1-002	AmpAmcFoxKf	*strB*
MET A1-003	SfSxtCCnKSCipNAmpT	*aadA1, aadA2, aphA1-IAB,* bla_{TEM-1}, *dhfrI, flo, strA, strB, sul1, sul2, tetA*
MET A1-004	CipN	-
MET A1-005	SfSxtCnKCipNAmpT	*aadA1, aadA2, aphA1-IAB,* bla_{TEM-1}, *tetA*
MET A1-007	SAmpKf	*aadA1,* bla_{TEM-1}
MET A1-008	SfSxtCKSCipNAmpTKf	*aphA1-IAB,* bla_{TEM-1}, *cat1, strA, strB, sul2*
MET A1-009	Kf	*strB*
MET A1-010	SfSxtCCnSCipNAmpT	*aadA1,* bla_{TEM-1}, *dhfrI, flo, strB, sul2, tetA*
MET A1-011	SfSCipNT	*strA, strB, sul1, tetA*
MET A1-012	SfKCipNT	*aadA1, aadA2, tetA*
MET A1-014	Susceptible	*aadA2, strB*
MET A1-015	SfSxtCSCipNAmpT	*aadA1, aadA2, aphA1-IAB,* bla_{TEM-1}, *flo, strB, sul1, sul2, tetA*
MET A1-016	SfSxtCCnSCipNAmpTKf	*aadA1, aphA1-IAB, dhfrI, flo, strB, sul1, sul2, tetA*
MET A1-017	Susceptible	*aadA1,* bla_{TEM-1}
MET A1-018	Susceptible	*aphA1-IAB,* $bla_{TEM-1,}$ bla_{CMY-2}
MET A1-019	SfSxtCKSCipNAmpTKf	*aphA1-IAB, cat1, strB, sul2*
MET A1-020	SfT	*tetA*
MET A1-021	CipNEtp	*aadA2,* bla_{TEM-1}

*Cro: ceftriaxone, Eft: ceftiofur, Imp: imipenem, Ak: amikacin, Cn: gentamicin, Amc: amoxicillin-clavulanic acid, Fox: cefoxitin, Etp: ertapenem, S: streptomycin, Sf: sulfisoxazole, Amp: ampicillin, Sxt: trimethoprim/sulfamethoxazole, K: kanamycin, C: chloramphenicol, Cip: ciprofloxacin, Kf: cephalotin, N: nalidixic acid, T: tetracycline.

tetracycline, nalidixic acid, ciprofloxacin, and levofloxacin (62). Moreover, *E. coli* isolated from chicken-based ready-to-eat foods demonstrated commonly resistance to tetracycline, ampicillin, and chloramphenicol in Singapore (61). Furthermore, *E. coli* isolates from local chicken meat products were considerably resistant to tetracycline, sulphonamide, ampicillin, and trimethoprim compared to imported chicken meat in Ghana (63), while in China *E. coli* isolated from chickens indicated resistance to oxytetracycline, amoxicillin, doxycycline, lomefloxacin, ceftriaxome, ofloxacin, enrofloxacin, and florfenicol, which is a distinct phenotypic antibiotic resistance profile in some degree compared to the former ones (64). In addition to the phenotypic characterization, the genotypic antibiotic resistance profiles of *E. coli* isolates, collected in this study, were determined as *strB* (52%), *aadA1* (42%), *tetA* (42%), *aphA1-IAB* (36%), bla_{TEM-1} (36%), *sul2* (31%), *aadA2* (31%), *sul1* (21%), *dhfrI* (15%), *flo* (15%), *strA* (15%), bla_{CMY-2} (10%), *cat1* (10%), and *ampC* (5%). On the other hand, bla_{PS1E-1}, *cat2*, *cmlA*, *aacC2*, *dhfrXII*, *tetB*, and *tetG* were not found in commensal *E. coli* isolates. Although *E. coli* isolates showed high resistance to ciprofloxacin, nalidixic acid, and tetracycline in phenotypic level, *strB* and *aadA1* conferring resistance to streptomycin, and *tetA* for tetracycline were mostly observed in genotypic level. In other words, in some *E. coli* strains, phenotypic and genotypic profiles could not be detected as compatible with each other. This might be that the primers used in this study were picked mainly for *Salmonella* isolates. Hence, mutations on primer binding regions might inhibit the detection. In the literature, bla_{CTX-M}, bla_{TEM-1}, *aadA1*, *tetA*, and *tetB* were detected in CTX-M-type extended spectrum β-lactamase-producing *E. coli* isolated from chickens in Great Britain (65). Furthermore, bla_{CMY-2}, *tetA*, *sul1*, *aac(3)-VIa*, and *ant(3")-Ia* were determined in extended-spectrum beta-lactamase (ESBL)/plasmidic AmpC (pAmpC) producing *E. coli* isolated from broiler parent birds in Finland (66). pAmpC β-lactamases conferring resistance to extended-spectrum cephalosporins in *Enterobacteriaceae,* especially in *E. coli*, have become threat for humans and livestock isolates (67,68). Genes of TEM, CTX-M and SHV families are most prevalent ones (69). Additionally, CMY-2 is the most predominant pAmpC in *E. coli* derived from distinct continents including Asia, North America, and Europe (61). In addition to this, CMY-2 has recently been determined in *Salmonella* and *E. coli* isolates from different types of food animals (61). In this study, bla_{CMY-2} was found in two *E. coli* isolates (i.e. MET A1-001 and -018), while it was not determined in *Salmonella* Infantis isolates. Although *E. coli* isolates were commensal bacteria, they demonstrated a great variety of antibiotic resistance compared to *Salmonella* isolates. Intestinal tract of humans and animals host numerous bacterial species and distinct serovars (70). Commensal bacteria perform some life-sustaining biological functions in the gastrointestinal tract (71). However, usage and/or misusage of antibiotics in human and veterinary for a long time affect normal gut microbiota adversely (72). Moreover, commensal bacteria might acquire antibiotic resistance genes from pathogenic bacteria via conjugative transfer such as plasmids (73). Hence, our commensal *E. coli* isolated from raw chicken products might possess multidrug resistance on a large scale due to high conjugative rate of mobile genetic elements. Apart from phenotypic antimicrobial resistance profile, in genotypic profiles of *Salmonella* isolates, except for nalidixic acid, antimicrobial resistance genes related to phenotypic resistance profile were detected using purified DNA. In *E. coli* isolates, various genotypic antibiotic resistance profiles were found because of presence of different strains.

4. Conclusion

To conclude, *Salmonella* Infantis isolated from raw chicken products in Turkey indicated closely related phenotypic and genotypic antimicrobial resistance profile including tetracycline, streptomycin, kanamycin, sulfisoxazole, and nalidixic acid with other Infantis clones found in different countries and/or continents. However, *E. coli* isolates were diversified. Globalization in food trading might lead Infantis to be an emerging strain. Furthermore, this study revealed that the intestines of poultry might be a gene pool for the commensal bacteria to acquire antibiotic resistance.

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References

1. Thorns CJ. Bacterial food-borne zoonoses. Revue Scientifique et Technique (International Office of Epizootics) 2000; 19 (1): 226-239.

2. Zaidi MB, Campos FD, Estrada-García T, Gutierrez F, León M et al. Burden and transmission of zoonotic foodborne disease in a rural community in Mexico. Clinical Infectious Diseases 2012; 55 (1): 51-60. doi: 10.1093/cid/cis300

3. Basler C, Nguyen TA, Anderson TC, Hancock T, Behravesh CB. Outbreaks of human *Salmonella* infections associated with live poultry, United States, 1990-2014. Emerging Infectious Diseases 2016; 22 (10): 1705-1711. doi: 10.3201/eid2210.150765

4. Dho-Moulin M, Fairbrother JM. Avian pathogenic *Escherichia coli* (APEC). Veterinary Research 1999; 30: 299-316.

5. European Food Safety Authority. Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Europe: Taking Stock. EFSA Journal 2011; 9 (10): 2390.

6. Santos RL, Zhang S, Tsolis RM, Kingsley RA, Adams GL et al. Animal models of *Salmonella* infections: Enteritis versus typhoid fever. Microbes and Infection 2001; 3 (14-15): 1335-1344. doi: 10.1016/S1286-4579(01)01495-2

7. European Food Safety Authority. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA Journal 2016; 14 (12): 231. doi: 10.2903/j.efsa.2016.4634

8. USDA-FSIS (2017). Serotypes profile of *Salmonella* isolates from meat and poultry products January 1998 through December 2014 [online]. Website https://www.fsis.usda.gov/wps/wcm/connect/3866026a-582d-4f0e-a8ce-851b39c7390f/Salmonella-Serotype-Annual-2014.pdf?MOD=AJPERES. [accessed 19 March 2019].

9. European Food Safety Authority, European Centre for Disease Prevention and Control. The European Union Summary Report on antimicrobial resistance in Antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in the European Union in 2015. EFSA Journal 2017; 15 (2): 4694.

10. Centers for Disease Control and Prevention.National Enteric Disease Surveillance: *Salmonella* Annual Report, 2016. Georgia, GA, USA: National Center for Emerging and Zoonotic Infectious Diseases; 2018.

11. Gal-Mor O, Valinsky L, Weinberger M, Guy S, Jaffe J et al. Multidrug-resistant *Salmonella enterica* serovar Infantis, Israel. Emerging Infectious Diseases 2010; 16 (11): 1754-1757. doi: 10.3201/eid1611.100100

12. Barco L, Barrucci F, Olsen JE, Ricci A. *Salmonella* source attribution based on microbial subtyping. International Journal of Food Microbiology 2013; 163 (2-3): 193-203. doi: 10.1016/j.ijfoodmicro.2013.03.005

13. Fratamico PM, DebRoy C, Liu Y, Needleman DS, Baranzoni GM et al. Advances in molecular serotyping and subtyping of *Escherichia coli*. Frontiers in Microbiology 2016; 7: 644. doi: 10.3389/fmicb.2016.00644

14. Zheng J, Keys CE, Zhao S, Ahmed R, Meng J et al. Simultaneous analysis of multiple enzymes increases accuracy of pulsed-field gel electrophoresis in assigning genetic relationships among homogeneous *Salmonella* strains. Journal of Clinical Microbiology 2011; 49 (1): 85-94. doi: 10.1128/JCM.00120-10

15. Thong KL, Ngeow YF, Altwegg M, Navaratnam P, Pang T. Molecular analysis of *Salmonella*Enteritidis by pulsed-field gel electrophoresis and ribotyping. Journal of Clinical Microbiology 1995; 33 (5): 1070-1074.

16. Li W, Raoult D, Fournier PE. Bacterial strain typing in the genomic era. FEMS Microbiology Reviews 2009; 33 (5): 892-916. doi: 10.1111/j.1574-6976.2009.00182.x

17. Harbottle H, White DG, McDermott PF, Walker RD, Zhao S. Comparison of multilocus sequence typing, pulsed-field gel electrophoresis, and antimicrobial susceptibility typing for characterization of *Salmonella enterica* serotype Newport isolates. Journal of Clinical Microbiology 2006; 44 (7): 2449-2457. doi:10.1128/JCM.00019-06

18. Eichhorn I, Heidemanns K, Semmler T, Kinnemann B, Mellmann A et al. Highly virulent non-O157 enterohemorrhagic *Escherichia coli* (EHEC) serotypes reflect similar phylogenetic lineages, providing new insights into the evolution of EHEC. Applied and Environmental Microbiology 2015; 81 (20): 7041-7047. doi: 10.1128/AEM.01921-15

19. Kim JS, Lee GG, Park JS, Jung YH, Kwak HS et al. A novel multiplex PCR assay for rapid and simultaneous detection of five pathogenic bacteria: *Escherichia coli* O157:H7, *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Vibrio parahaemolyticus*. Journal of Food Protection 2007; 70 (7): 1656-1662.

20. Osés SM, Rantsiou K, Cocolin L, Jaime I, Rovira J. Prevalence and quantification of Shiga-toxin producing *Escherichia coli* along the lamb food chain by quantitative PCR. International Journal of Food Microbiology 2010; 141: 163-169. doi: 10.1016/j.ijfoodmicro.2010.05.010

21. Wang L, Rothemund D, Curd H, Reeves PR. Sequence diversity of the *Escherichia coli* H7 fliC genes: Implication for a DNA-based typing scheme for *E. coli* O157:H7. Journal of Clinical Microbiology 2000; 38 (5): 1786-1790.

22. Bai J, Shi X, Nagaraja TG. A multiplex PCR procedure for the detection of six major virulence genes in *Escherichia coli* O157:H7. Journal of Microbiological Methods 2010; 82 (1): 85-89. doi:10.1016/j.mimet.2010.05.003

23. Fagan PK, Hornitzky MA, Bettelheim KA, Djordjevic SP. Detection of shiga-like toxin (stx1 and stx2), intimin (eaeA), and enterohemorrhagic *Escherichia coli* (EHEC) hemolysin (EHEC hlyA) genes in animal feces by multiplex PCR. Applied and Environmental Microbiology 1999; 65 (2): 868-872.

24. Bertrand R, Roig B. Evaluation of enrichment-free PCR-based detection on the rfbE gene of *Escherichia coli* O157-Application to municipal wastewater. Water Research 2007; 41 (6): 1280-1286. doi: 10.1016/j.watres.2006.11.027

25. Botkin DJ, Galli L, Sankarapani V, Soler M, Rivas M et al. Development of a Multiplex PCR Assay for Detection of Shiga Toxin-Producing *Escherichia coli, Enterohemorrhagic E. coli, and Enteropathogenic E. coli* Strains. Frontiers in Cellular and Infection Microbiology 2012; 2: 1-10. doi: 10.3389/fcimb.2012.00008

26. Presterl E, Zwick RH, Reichmann S, Aichelburg A, Winkler S et al. Frequency and virulence properties of diarrheagenic *Escherichia coli* in children with diarrhea in Gabon. American Journal of Tropical Medicine and Hygiene 2003; 69 (4): 406-410.

27. Stacy-Phipps S, Mecca JJ, Weiss JB. Multiplex PCR assay and simple preparation method for stool specimens detect enterotoxigenic *Escherichia coli* DNA during course of infection. Journal of Clinical Microbiology 1995; 33 (5): 1054-1059.

28. Guion CE, Ochoa TJ, Walker CM, Barletta F, Cleary TG. Detection of diarrheagenic *Escherichia coli* by use of melting-curve analysis and real-time multiplex PCR. Journal of Clinical Microbiology 2008; 46 (5): 1752-1757. doi: 10.1128/JCM.02341-07

29. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB et al. Standardization of Pulsed-Field Gel Electrophoresis Protocols for the Subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathogens and Disease 2006; 3 (1): 59-67. doi:10.1089/fpd.2006.3.59

30. Harbottle H, White DG, McDermott PF, Walker RD, Zhao S. Comparison of multilocus sequence typing, pulsed-field gel electrophoresis, and antimicrobial susceptibility typing for characterization of *Salmonella enterica* serotype Newport isolates. Journal of Clinical Microbiology 2006; 44 (7): 2449-2457. doi:10.1128/JCM.00019-06

31. Steenackers H, Hermans K, Vanderleyden J, De Keersmaecker SCJ. Salmonella biofilms: An overview on occurrence, structure, regulation and eradication. Food Research International 2012; 45 (2): 502-531. doi: 10.1016/j.foodres.2011.01.038

32. Pavlickova S, Klancnik A, Dolezalova M, Mozina SS, Holko I. Antibiotic resistance, virulence factors and biofilm formation ability in *Escherichia coli* strains isolated from chicken meat and wildlife in the Czech Republic. Journal of Environmental Science and Health- Part B Pesticides, Food Contaminants, and Agricultural Wastes 2017; 52 (8): 570-576. doi: 10.1080/03601234.2017.1318637

33. Rossi DA, Melo RT, Mendonça EP, Monteiro GP. Biofilms of *Salmonella* and *Campylobacter* in the poultry industry. Poultry Science InTech 2017; 93-113. doi: 10.5772/65254

34. Stepanović S, Ćirković I, Ranin L, Švabić-Vlahović M. Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. Letters in Applied Microbiology 2004; 38 (5): 428-432. doi: 10.1111/j.1472-765X.2004.01513.x

35. CLSI. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. Approved Standard—Second Edition 2002; 22 (6): M31-A2.

36. CLSI. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing. Twenty-First Informational Supplement 2011; 31 (1): M100-S21.

37. Chen S, Zhao S, White DG, Schroeder CM, Lu R et al. Characterization of Multiple-Antimicrobial-Resistant *Salmonella* Serovars Isolated from Retail Meats. Applied and Environmental Microbiology 2004; 70 (1): 1-7.

38. Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. Journal of Clinical Microbiology 2002; 40 (6): 2153-2162.

39. Randall LP, Cooles SW, Osborn MK, Piddock LJV, Woodward MJ. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. Journal of Antimicrobial Chemotherapy 2004; 53 (2): 208-216. doi: 10.1093/jac/dkh070

40. Gebreyes WA, Altier C. Molecular Characterization of multidrug-resistant *Salmonella enterica* subsp. *enterica* serovar typhimurium isolates from Swine. Journal of Clinical Microbiology 2002; 40 (8): 2813-2822.

41. Frana TS, Carlson SA, Griffith RW. Relative distribution and conservation of genes encoding aminoglycoside-modifying enzymes in *Salmonella enterica* serotype typhimurium phage type DT104. Applied and Environmental Microbiology 2001; 67 (1): 445-448. doi: 10.1128/AEM.67.1.445-448.2001

42. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*, review. Nature Reviews Microbiology 2004; 2 (2): 123-140. doi: 10.1038/nrmicro818

43. Allocati N, Masulli M, Alexeyev MF, Di Ilio C. Escherichia coli in Europe: An overview. International Journal of Environmental Research and Public Health 2013; 10 (12): 6235-6254. doi: 10.3390/ijerph10126235

44. Farrokh C, Jordan K, Auvray F, Glass K, Oppegaard H et al. Review of Shiga-toxin-producing Escherichia coli (STEC) and their significance in dairy production. International Journal of Food Microbiology 2013; 162 (2): 190-212. doi: 10.1016/j.ijfoodmicro.2012.08.008

45. Almeida F, Pitondo-Silva A, Oliveira MA, Falcão JP. Molecular epidemiology and virulence markers of *Salmonella* Infantis isolated over 25 years in São Paulo State, Brazil. Journal of Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases 2013; 19: 145-151. doi: 10.1016/j.meegid.2013.07.004

46. Ed-Dra A, Karraouan B, Allaoui AE, Khayatti M, Ossmani HE et al. Isolation, antimicrobial resistance and genetic diversity of *Salmonella* Infantis isolates from foods and human samples in Morocco. Journal of Global Antimicrobial Resistance 2018; 14: 297-301. doi: 10.1016/j.jgar.2018.05.019

47. Nógrády N, Király M, Davies R, Nagy B. Multidrug resistant clones of *Salmonella* Infantis of broiler origin in Europe. International Journal of Food Microbiology 2012; 157 (1): 108-112. doi:10.1016/j.ijfoodmicro.2012.04.007

48. Shahada F, Sugiyama H, Chuma T, Sueyoshi M, Okamoto K. Genetic analysis of multi-drug resistance and the clonal dissemination of β-lactam resistance in *Salmonella* Infantis isolated from broilers. Veterinary Microbiology 2010; 140 (1-2): 136-141. doi: 10.1016/j.vetmic.2009.07.007

49. Miller T, Prager R, Rabsch W, Fehlhaber K, Voss M. Epidemiological relationship between *Salmonella* Infantis isolates of human and broiler origin. Lohmann Information 2010; 2: 27-31.

50. Nógrády N, Tóth Á, Kostyák Á, Pászti J, Nagy B. Emergence of multidrug-resistant clones of *Salmonella* Infantis in broiler chickens and humans in Hungary. Journal of Antimicrobial Chemotherapy 2007; 60 (3): 645-648. doi: 10.1093/jac/dkm249

51. Murgia M, Bouchrif B, Timinouni M, Al-Qahtani A, Al-Ahdal MN et al. Antibiotic resistance determinants and genetic analysis of *Salmonella enterica* isolated from food in Morocco. International Journal of Food Microbiology 2015; 215: 31-39. doi: 10.1016/j.ijfoodmicro.2015.08.003

52. Ceyssens PJ, Mattheus W, Vanhoof R, Bertrand S. Trends in serotype distribution and antimicrobial susceptibility in *Salmonella enterica* isolates from humans in Belgium, 2009 to 2013. Antimicrobial Agents and Chemotherapy 2015; 59 (1): 544-552. doi:10.1128/AAC.04203-14

53. Liang Z, Ke B, Deng X, Liang J, Ran L et al. Serotypes, seasonal trends, and antibiotic resistance of non-typhoidal *Salmonella* from human patients in Guangdong Province, China, 2009-2012. BMC Infectious Diseases 2015; 15 (1): 53. doi: 10.1186/s12879-015-0784-4

54. Zhu Y, Lai H, Zou L, Yin S, Wang C et al. Antimicrobial resistance and resistance genes in *Salmonella* strains isolated from broiler chickens along the slaughtering process in China. International Journal of Food Microbiology 2017; 259: 43-51. doi: 10.1016/j.ijfoodmicro.2017.07.023

55. Shigemura H, Matsui M, Sekizuka T, Onozuka D, Noda T et al. Decrease in the prevalence of extended-spectrum cephalosporin-resistant *Salmonella* following cessation of ceftiofur use by the Japanese poultry industry. International Journal of Food Microbiology 2018; 274: 45-51. doi: 10.1016/j.ijfoodmicro.2018.03.011

56. Dionisi AM, Lucarelli C, Benedetti I, Owczarek S, Luzzi I. Molecular characterisation of multidrug-resistant *Salmonella enterica* serotype Infantis from humans, animals and the environment in Italy. International Journal of Antimicrobial Agents 2011; 38 (5): 384-389. doi: 10.1016/j.ijantimicag.2011.07.001

57. Chuah LO, Shamila Syuhada AK, Mohamad Suhaimi I, Farah Hanim T, Rusul G. Genetic relatedness, antimicrobial resistance and biofilm formation of *Salmonella* isolated from naturally contaminated poultry and their processing environment in northern Malaysia. Food Research International 2018; 105: 743-751. doi: 10.1016/j.foodres.2017.11.066

58. Papadopoulos T, Petridou E, Zdragas A, Mandilara G, Vafeas G et al. Multiple clones and low antimicrobial resistance rates for *Salmonella enterica* serovar Infantis populations in Greece. Comparative Immunology, Microbiology and Infectious Diseases 2017; 51: 54-58. doi: 10.1016/j.cimid.2017.02.002

59. Raffatellu M, Tukel C, Chessa D, Wilson RP, Baumler AJ. The intestinal phase of *Salmonella* infections. In: Rhen M, Maskell D, Mastroeni P, Threlfall EJ, editor. *Salmonella*: Molecular Biology and Pathogenesis. Norfolk, United Kingdom: Horizon Bioscience; 2007. pp. 30-51.

60. Pasquali F, Lucchi A, Braggio S, Giovanardi D, Franchini A et al. Genetic diversity of *Escherichia coli* isolates of animal and environmental origins from an integrated poultry production chain. Veterinary Microbiology 2015; 178 (3-4): 230-237. doi: 10.1016/j.vetmic.2015.05.007

61. Guo S, Tay MYF, Aung KT, Seow KLG, Ng LC et al. Phenotypic and genotypic characterization of antimicrobial resistant *Escherichia coli* isolated from ready-to-eat food in Singapore using disk diffusion, broth microdilution and whole genome sequencing methods. Food Control 2019; 99: 89-97. doi: 10.1016/j.foodcont.2018.12.043

62. Ferreira JC, Penha Filho RAC, Andrade LN, Berchieri Junior A, Darini ALC. Evaluation and characterization of plasmids carrying CTX-M genes in a non-clonal population of multidrug-resistant Enterobacteriaceae isolated from poultry in Brazil. Diagnostic Microbiology and Infectious Disease 2016; 85 (4): 444-448. doi: 10.1016/j.diagmicrobio.2016.05.011

63. Rasmussen MM, Opintan JA, Frimodt- Møller N, Styrishave B. Beta-lactamase producing *Escherichia coli* isolates in imported and locally produced chicken meat from Ghana. PLoS ONE 2015; 10 (10): e0139706. doi: 10.1371/journal.pone.0139706

64. Li L, Wang B, Feng S, Li J, Wu C et al. Prevalence and characteristics of extended-spectrum β-lactamase and plasmid-mediated fluoroquinolone resistance genes in *Escherichia coli* isolated from chickens in Anhui Province, China. PLoS ONE 2014; 9 (8): e104356. doi: 10.1371/journal.pone.0104356

65. Toszeghy M, Phillips N, Reeves H, Wu G, Teale C et al. Molecular and phenotypic characterisation of Extended Spectrum β-lactamase CTX-M *Escherichia coli* from farm animals in Great Britain. Research in Veterinary Science 2012; 93 (3): 1142-1150. doi: 10.1016/j.rvsc.2012.05.001

66. Oikarainen PE, Pohjola LK, Pietola ES, Heikinheimo A. Direct vertical transmission of ESBL/pAmpC-producing *Escherichia coli* limited in poultry production pyramid. Veterinary Microbiology 2019; 231: 100-106. doi: 10.1016/j.vetmic.2019.03.001

67. Bush K. Alarming beta-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Current Opinion in Microbiology 2010; 13 (5): 558-564. doi: 10.1016/j.mib.2010.09.006

68. Liebana E, Carattoli A, Coque TM, Hasman H, Magiorakos AP, et al. Public health risks of enterobacterial isolates producing extended-spectrum β-lactamases or AmpC β-lactamases in food and food-producing animals: an EU perspective of epidemiology, analytical methods, risk factors, and control Options. Clinical Infectious Diseases 2013; 56 (7): 1030-1037. doi: 10.1093/cid/cis1043

69. Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH. Extended-spectrum beta-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clinical Microbiology and Infection 2012; 18 (7): 646-655. doi: 10.1111/j.1469-0691.2012.03850.x

70. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science 2005; 307: 1915-1920. doi: 10.1126/science.1104816

71. Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology 2010; 156: 3216-3223. doi: 10.1099/mic.0.040618-0

72. Casals-Pascual C, Vergara A, Vila J. Intestinal microbiota and antibiotic resistance: Perspectives and solutions. Human Microbiome Journal 2018; 37: 1-27. doi: 10.1016/j.humic.2018.05.002

73. Penders J, Stobberingh EE, Savelkoul PHM, Wolffs PFG. The human microbiome as a reservoir of antimicrobial resistance. Frontiers in Microbiology 2013; 4: 87. doi: 10.3389/fmicb.2013.00087