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Phenotyping and genetic characterization of *Salmonella enterica* isolates from Turkey revealing arise of different features specific to geography☆

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ABSTRACT

192 Food samples (commonly consumed 8 food types), 355 animal samples (animal feces of bovine, ovine, goat and chicken) and 50 samples from clinical human cases in Sanliurfa city, Turkey in a year were collected to determine the *Salmonella enterica* subsp. *enterica* mosaic in Turkey. 161 *Salmonella* isolates represented 17 serotypes, 20 sequence types (STs) and 44 PFGE patterns (PTs). 3 serotypes, *S.* Enteritidis, *S.* Typhimurium and *S.* Kentucky, were recovered from three different hosts. The highest discriminatory power was obtained by PFGE (SID = 0.945), followed by MLST (SID = 0.902) and serotyping (SID = 0.885) for all isolates. The prevalence of antimicrobial resistance genes (*aadA1*, *aadA2*, *strA*, *strB*, $aphA_{1\text{-}Iab}$, $bla_{TEM\text{-}1}$, $bla_{PSE\text{-}1}$, *tetA*) was highly correlated with phenotypic profiles of aminoglycoside, ß-lactam and tetracycline groups (kappa >0.85). From our knowledge, this is the first study reporting spatial and temporal distribution of *Salmonella* species through phenotypic and genetic approaches over farm to fork chain in Turkey. Thus, our data provided further information for evolution, ecology and transmission of *Salmonella* in Turkey.

1. Introduction

Salmonella enterica subsp. *enterica*, belonging to Enterobacteriaceae, is a gram-negative, zero-tolerant, rod shaped, facultatively anaerobic bacterium. Salmonellosis, caused by nontyphoidal serotypes, is a critical medical problem that causes symptoms of gastroenteritis including diarrhea, nausea, abdominal pain, vomiting, mild fever and chills. The number of salmonellosis infections reaches up to approximately 93.8 million infections for each year worldwide (Majowicz et al., 2010). Typhoidal *Salmonella* serotypes, such as Typhi and Paratyphi A, B and C, on the other hand, may initiate enteric fever. Especially, *S.* serovar Paratyphi A has recently begun to take over *S.* serovar Typhi as the main agent of enteric fever in many Asian countries (Teh et al., 2014). Thus, global progressive increase of paratyphoid fever worldwide (Ochiai et al., 2005) has turned into a main health problem, especially in developing countries such as China and Pakistan (Girard et al., 2006).

Salmonellosis can include mild to severe symptoms in humans and animals, and in severe cases antimicrobial treatment is unavoidable. According to the recent studies, there is an increase in antimicrobial resistance (AR) among *Salmonella* isolates, due to use the (misuse) of antimicrobial drugs in human and veterinary medicine, causing a selective pressure for the proliferation of resistant bacteria (Foley and Lynne, 2008). The resistance profile may change depending on time, serotype, subtype, and source of microorganism and also geographic region of isolate.

Foodborne infections cause public health problems, thus understanding the nature of these diseases is very important (Herikstad et al., 2002). The phenotypic methods are still commonly used, and highly applied, because of the ease of their interpretation to detect and characterize pathogens. For example, although serotyping and phage typing are widely used methods, they have low discriminatory power and are time consuming compared to molecular subtyping methods (Boxrud et al., 2007). Differences among pathogens and further phenotypic characters can be detected by the subtyping methods such as ribotyping, pulsed field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), multilocus sequence typing (MLST) and multiple locus variable number tandem repeat analysis (MLVA). Among the subtyping methods, PFGE (Swaminathan et al., 2001), known as the gold standard for *Salmonella* typing (Barrett et al., 2006), is still the most widely used molecular method, whereas, MLST (Achtman et al., 2012; Maiden, 2006) is

☆ *Salmonella* diversity from farm to fork in Turkey.

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mostly used for the population structure studies and detecting genetically related clones.

To protect public health, *Salmonella* should be monitored from the farm/field to fork chain. Although Turkey is a major food producing country, there is a lack of surveillance system focusing on the farm to fork chain. The objective of this research was to determine *Salmonella* mosaic by sampling from food animals, humans and foods, at the population level using genotypic and phenotypic methods. In a wider perspective, our objective was to develop a reference surveillance system on foodborne pathogens that can be used by health authorities as well as researchers worldwide in a pilot region, Sanliurfa region, Turkey. For this aim, *Salmonella* isolates that were collected from street foods, as well as from animal feces and human clinical cases in Sanliurfa region were further characterized by phenotypic (serotyping, disk diffusion) and genotypic (MLST, PFGE and AR gene profiling) methods. Isolate information of phenotypic and genotypic characterizations was downloaded from publicly available websites (Food Microbe Tracker: http://www.foodmicrobetracker.com/login/login.aspx; Pathogen Detector: pathogendetector-metu.rhcloud.com).

2. Materials and methods

2.1. Food isolates

All isolates were obtained from Sanliurfa, Southeast Anatolian Region of Turkey. From April 2012 to January 2013, food samples were collected from eight different food types: (i) ground lamb, (ii) ground beef, (iii) chicken meat, (iv) unripened cheese, (v) Urfa (ripened) cheese, (vi) pistachio, (vii) pepper and (viii) isot (paprika). Samples were collected from two different locations and three different quality types, which was determined according to their prices. In each season (summer, autumn, winter and spring) 48 samples (8 type × 2 location × 3 quality type) were collected. All food samples were transported on ice to the Middle East Technical University (METU) Food Engineering Department (Ankara, Turkey) overnight for *Salmonella* detection and isolation, as well as characterization. A total 192 samples were studied for *Salmonella* isolation according to ISO 6579 procedure in METU, Ankara (Durul et al., 2015).

2.2. Animal isolates

For each season, from April 2012 to January 2013, 355 fecal samples were collected from clinical animal cases in the Animal Hospital of Veterinary Faculty, Harran University. Moreover, fecal samples were collected from poultry, bovine and, sheep farms and also from slaughterhouses. Overall, 83 animal-related isolates were collected from chicken, cow, sheep and goat fecal samples according to ISO6579 procedure in Harran University, Sanliurfa and collected suspicious *Salmonella* isolates were sent to METU in *Salmonella Shigella* (SS) agar on ice for confirmation and further studies.

2.3. Clinical human isolates

Fecal and/or blood samples were taken from patients with salmonellosis or suspected of having salmonellosis diagnosis and the samples were collected in the Medicine Faculty of Harran University for four seasons during April 2012 to January 2013. Various methods were applied for the two different sample types.

Fecal samples were inoculated into blood agar, eosin methylene blue (EMB) agar and SS agar sequentially. Lactose negatives colonies in SS agar were then taken for biochemical tests. Suspicious colonies were inoculated into Simmons' citrate agar, urea agar, triple sugar iron (TSI) agar and also motility agar to characterize the isolates according to their citrate, urea, iron and motility properties (Davis and Morishita, 2005).

Blood samples, on the other hand, were directly taken in BD BACTEC 9050 Blood Culture System (BD Diagnostics, New Jersey, U.S.) in sterile conditions. The colonies were incubated on EMB, blood and chocolate agar. Lactose negative colonies were further analyzed according to the methods mentioned above.

A total of 50 presumptive *Salmonella* isolates were transported to METU on ice for further confirmation and characterization.

2.4. Molecular confirmation of suspicious Salmonella isolates from different sources

The molecular confirmation of all suspicious *Salmonella* isolates was conducted by investigating the presence of the *invA* gene (F: GAACCCTCAGTTTTTCAACGTTTC, R: TAGCCGTAACAACCAATACAAATG) by PCR. All confirmed *Salmonella* isolates (Supplementary Table 1) were stored at −80 °C under METU IDs in 15% glycerol for future studies in METU (Kim et al., 2007).

2.5. Serotyping

Serotyping of isolates was conducted according to White-Kauffmann-Le Minor Scheme (Grimont and Weil, 2007) at the laboratory of Public Health Agency of Turkey in Ankara, where the second *Salmonella* confirmation by using biochemical tests was also performed.

2.6. Multilocus sequence typing (MLST)

PCR amplification and the subsequent DNA sequencing processes were performed according to *Salmonella enterica* MLST at the University of Warwick (UoW) (available on http://mlst.warwick.ac.uk/mlst/). DNA fragments from seven house-keeping genes, *aroC* (639 nt), *dnaN* (833 nt), *hemD* (666 nt), *hisD* (894 nt), *purE* (510 nt), *sucA* (643 nt), *thrA* (852 nt) were used for MLST characterization. Genomic DNA isolation of the isolates was conducted via Nanobiz Bacterial Genomic DNA Isolation Kit (NANObiz, Ankara, Turkey). Purification of PCR products and DNA sequencing were conducted by Macrogen Inc. (Geumchon-gu, Seoul, Korea). All sequences were trimmed, proofread and assembled by using SeqMan and SeqBuilder software (DNAStar, Madison, USA). In accordance with the UoW MLST Database, trimmed sequences of certain length from *aroC* (501 nt), *dnaN* (501 nt), *hemD* (432 nt), *hisD* (501 nt), *purE* (399 nt), *sucA* (501 nt), *thrA* (501 nt) were aligned by Clustal W algorithm using MegAlign software (DNAStar, Madison, USA). Assignment of gene alleles was implemented in compliance with the allelic numbers specified in the UoW MLST Database. As the combination of seven allelic types, allelic type profiles of the isolates were formed. According to this profile information, same sequence type (ST) numbers were assigned for the isolates sharing the same allelic profiles of seven genes.

2.7. Pulsed-field gel electrophoresis (PFGE)

The PulseNet standardized protocol was used for *Salmonella* subtyping with the addition of 10 μM thiourea to the running buffer (Murase et al., 2004; Ribot et al., 2006). The DNA was digested with the restriction enzyme *Xba*I (Roche Applied Science, Germany) and *Salmonella* Braenderup H9812 was used as a molecular size standard in all PFGE investigations. Running parameters of the electrophoresis performed with the CHEF-DR III system electrophoresis cell (Bio-Rad Laboratories, CA, USA) were as follows: initial switch time-2.2 s, final switch time-63.8 s, voltage-6 V, time-19 h and temperature 14 °C.

2.8. Data analysis

Quantity One analysis (Bi-Rad Laboratories, CA, USA) software and Molecular Imager Gel Doc XR System Universal Hood II (Bio-Rad Laboratories, CA, USA) were used together to visualize PFGE gel pictures.

DNA bands were investigated to build the dendrograms using BioNumerics software (Applied Maths, Belgium). Similarity analysis was conducted using Dice coefficient and clustering was performed using the unweighted pair group method by arithmetic mean (UPGMA). Dice's similarity coefficient was used to compute the similarity of each banding pattern with a 1.5% band position tolerance (i.e., allowed relative distance that a single band within a lane can shift during aligning of the bands) and 1.5% optimization (i.e., allowed relative distance that a lane with all the bands can shift while matching the bands) (Ferris et al., 2004). PFGE and MLST types were assigned unique numerical identifiers (i.e., PT 1 and ST1, respectively).

2.9. Phenotypic and genotypic antimicrobial resistance profiling

Phenotypic antimicrobial resistance tests were done by the disk diffusion method (Lee et al., 1994). Eighteen different antimicrobial elements are studied: amikacin 30 μg (Ak), gentamicin 10 μg (Gn), kanamycin 30 μg (K), streptomycin 10 μg (S), ciprofloxacin 5 μg (CIP), nalidixic acid 30 μg (N), ampicillin 10 μg (Amp), amoxicillin-clavulanic acid 20/10 μg (Amc), tetracycline 30 μg (T), cefoxitin 30 μg (Fox), cephalothin 30 μg (Kf), ertapenem 10 μg (Etp), ceftriaxone 30 μg (Cro), ceftiofur 30 μg (Eft), sulfisoxazole (Sf), sulfamethoxazole-trimethoprim (Sxt), chloramphenicol (C), imipenem 10 μg (Ipm). The quality control strain was *E. coli* ATCC 25922 for the tests. Clinical Laboratory Standards Institute (CLSI, 2012) and the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2012) standards were used to determine the limits of resistance.

AR gene profiling was performed on the phenotypically resistant isolates. 21 antimicrobial resistance coding genes (*bla*$_{TEM-1}$, *bla*$_{PSE-1}$, *bla*$_{CMY-2}$, *ampC*, *cat1*, *cat2*, *flo*, *cmlA*, *aadA1*, *aadA2*, *strA*, *strB*, *aacC2*, *aphA*$_{1-Iab}$, *dhfrI*, *dhfrXII*, *sulI*, *sulII*, *tetA*, *tetB*, *tetG*) were amplified to determine genetic variation of AR (Soyer et al., 2013). The genes and the primers that were studied were given in Supplementary Table 2. *Salmonella* DNA were isolated prior to genotypic AR profiles analysis by DNA4U® Bacterial Genomic DNA Isolation Kit (Nanobiz, Ankara, Turkey).

2.10. Statistical methods

Relations between isolate source groups (i.e. human, food, animal), subgroups (i.e., food groups, animal species, gender) and resistance types (i.e., susceptible, intermediate, and resistant) were evaluated by Fisher's exact test. Analyses were carried out using R-project (www.r-project.org/).

The agreement of two studies; phenotypic and genotypic AR profiles; was determined by Kappa statistics in Minitab 17 Statistical Software (Minitab, Inc., State College, PA). Cohen's Kappa, which is a statistical measure of inter-rater agreement or inter-annotator agreement (Carletta, 1996) for qualitative items, was calculated according to the formula given below.

$$\kappa = \frac{P_r(a)\text{-}P_r(e)}{1\text{-}P_r(e)} \quad (1)$$

Pr(a) is the relative observed agreement among raters, and Pr(e) is the hypothetical probability of chance agreement, using the observed data to calculate the probabilities of each observer randomly saying each category. Kappa value 0 indicates that the tests agree as well as would be expected by chance and a value of 1 indicates complete agreement. Scores <0.20 = poor, 0.21–0.40 = fair, 0.41–0.60 = moderate, 0.61–0.80 = good and 0.81–1.00 = very good agreement. Kappa analysis is not performed for quinolone resistance group, since quinolone AR genotype is not involved in the study.

Odds ratio (OR) was used to determine the association of resistance genes that were significantly different with 95% confidence intervals (CI) (Altman, 1990). Bonferroni corrections were used as a conservative modification for multiple comparisons setting the level of statistical significance at $p < 0.05/n$, where n is the number of comparisons made for each outcome (Dohoo et al., 2009). An OR of >1 indicated a positive association between the outcome and predictor variable, while an OR of <1 indicated a negative association between the outcome and predictor variable.

3. Results and discussion

In our study, 59 food, 52 animal and 50 clinical human *Salmonella* isolates were obtained from 192 food, 355 animal, and 50 clinical human samples (Supplementary Table 1).

The distributions of *Salmonella* serotypes for 3 different sources were varied (Table 1), and the variations of serotypes for food and animal samples were high ($SID_{food,serotype} = 0.832$, $SID_{animal,serotype} = 0.821$, $SID_{human,serotype} = 0.567$). The most frequently observed serotypes were different in each sample groups; *S.* Infantis (25.8%), *S.* Montevideo (37.7%) and *S.* Paratyphi B (64.0%) were the most observed serotypes in food, animal and human *Salmonella* isolates, respectively. Although most of the animal isolates were obtained from the bovine group (61%), the dispersed diversity of *Salmonella* serotypes (n = 10) in ovine fecal samples was noteworthy.

Only two serotypes, *S.* Kentucky and *S.* Typhimurium were obtained from all three sources (Table 1). Notably, a rarely seen serotype worldwide, *S.* Othmarschen, had been isolated from the two sources; food and clinical human samples with 1.7% (1/59), and 4.0% (2/50), respectively.

3.1. Salmonella serotype distribution in farm to fork chain

In clinical human isolates, serotypes did not vary as we observed in food and animal isolates, only 6 different serotypes were detected. The parameters, such as location of the cases and gender, did not affect the serotype distribution in clinical human isolates (p-value >0.05), among which *S.* Paratyphi B was the most common serotype in city, Sanliurfa. This might be due to asymptomatic hosts or low hygienic conditions of the environment. Also, since the number of paratyphoid fever cases has increased and is higher than that of typhoid fever in developing countries, it is not surprising to observe *S.* Paratyphi B at a high prevalence rate in Sanliurfa city, which has very poor urban region around (Hawker et al., 2012). In the city center, besides *S.* Paratyphi B and few *S.* Typhi isolates, isolates representing nontyphodial serotypes such as *S.* Enteritidis, *S.* Kentucky, *S.* Othmarschen, and *S.* Typhimurium were also collected from human salmonellosis cases, most likely due to the contaminated food.

The common serotypes, found in this study, *S.* Montevideo (n = 29; 15.4%) and *S.* Telaviv (n = 22; 13%), had risen to notice, since neither *S.* Montevideo, nor *S.* Telaviv were commonly collected serotypes worldwide. Association of serotype *S.* Telaviv with bovine was reported both in Turkey and England previously (Erol, 1999; Richardson, 1975). In our study, *S.* Telaviv (ST 1068) was frequently found in a variety of foods (i.e., ground beef meat, ground lamb meat, unripened cheese, Urfa cheese) and food animals (i.e., bovine and ovine feces). Since it is not a dominant serotype in Europe and the United States, the prevalence of *S.* Telaviv in Turkey shows the possible emergence of this serotype in this geographic area, which was reported earlier (Durul et al., 2015).

Another noteworthy serotype was *S.* Infantis, which had been associated with chicken samples (chicken breast, chicken skin, and chicken wing). Among 21 isolates collected from chicken samples, 15 represented the serotype *S.* Infantis and these isolates dominated the number of isolates from all food samples (p-value <0.05); all the *S.* Infantis isolates were from chicken sources such as wings, skin, and breast. Similarly, European Food Safety Authority (EFSA) (ECDC, 2015) reports indicated that *S.* Infantis has been very common among breeding flocks (second order) and also human (fourth order). While this serotype was very persistent among food related sources, in our study it was not observed in animal and clinical human samples.

Table 1
Distribution of sequence type (ST), PFGE type (PT) and, antimicrobial resistance profile of 161 *Salmonella* isolates [(1)].

Subspecies	Serotype	Total number of isolates	Sequence type (ST)	PFGE Type (PT)	Antimicrobial resistance profile	Number of isolates from			Detailed source
						Food	Animal	Clinical human	
enterica	Infantis	**15**	**32**	**7**	**K-S-T-Sf-N**	2	–	–	**Chicken meat (wing, breast, liver,**
				8	**S-T-N**	1	–	–	**drumstick, offal)**
					S-T-Sf-N	5	–	–	
					K-S-T-Sf-N	4	–	–	
					K-S-T-Amp-Sf-N	1	–	–	
					K-S-T-Amp-Kf-Sf-Sxt-C-N	1	–	–	
				9	**S-T-Sf-N**	1	–	–	
	Telaviv	**22**	**1068**	**12**	Susceptible	–	1	–	Bovine feces
				33	Susceptible	9	1	–	Ground beef meat, ground lamb meat, cheese, offal, bovine feces
				34	Susceptible	3	3	–	Ground beef meat, cheese, bovine feces
				36	Susceptible	1	–	–	Cheese
				37	Susceptible	–	1	–	Ovine feces
				38	Susceptible	–	3	–	Bovine feces
	Anatum	**12**	**64**	**42**	**Ak-Sf**	–	1	–	**Ovine feces**
					Sf	3	–	–	**Ground lamb meat**
					Susceptible	8	–	–	Ground beef meat, ground lamb meat
	Montevideo	**29**	**195**	**19**	Susceptible	–	4	–	Bovine feces
					Sf	**1**	**1**	**–**	**Bovine feces, ground lamb meat**
				20	Susceptible	–	1	–	Ovine feces
				21	Susceptible	3	–	–	Ground lamb meat, offal
				22	Susceptible	2	–	–	Ground beef meat, offal
				24	Susceptible	–	2	–	Bovine feces
				25	Susceptible	3	2	–	Offal, ovine feces, bovine feces
				28	Susceptible	1	1	–	Bovine feces, ground beef meat
				44	Susceptible	–	1	–	Bovine feces
			138	**31**	**Fox-Kf-Etp**	–	1	–	**Bovine feces**
					Fox-Kf	–	1	–	**Bovine feces**
					T-Ert	–	1	–	**Bovine feces**
					Sf	–	2	–	**Chicken, bovine feces**
					Susceptible	–	2	–	Bovine feces
	Reading	**5**	**93**	**32**	Susceptible	2	–	–	Ground beef meat, offal
				17	**Amc-Fox-Kf-Etp**	–	1	–	**Ovine feces**
					Susceptible	1	–	–	Ground lamb meat
			1831	**43**	Susceptible	–	1	–	Ovine feces
	Newport	**4**	**166**	**40**	Susceptible	2	–	–	Ground beef meat, offal
			1822	**11**	Susceptible	–	1	–	Ovine feces
			31	**39**	**Sf**	–	1	–	**Bovine feces**
	Kentucky	**14**	**314**	**10**	**Sf**	1	–	4	**Human, ground meat**
					Susceptible	1	1	1	Bovine feces, offal, human
			1807	**3**	Susceptible	–	5	–	Bovine feces
					Sf	–	1	–	**Ovine feces**
	Hadar	**2**	**33**	**41**	**S-T-Amp-Amc-Fox-Kf-Etp-N**	–	1	–	**Ovine feces**
					S-T-Amp-Kf-N	1	–	–	**Cheese**
	Othmarschen	**3**	**1832**	**27**	Susceptible	1	–	–	Ground lamb meat
				29	**Sf**	–	–	1	**Human**
					Susceptible	–	–	1	Human
	Typhimurium	**11**	**19**	**13**	**Ak-S-T-Amp-Kf-N**	–	1	–	**Bull feces**
					T-Amp-Kf	–	1	–	**Ovine feces**
					T-Amp	1	–	2	**Human, offal**
				14	**S-T-Amp-Amc-Sf-C-N**	–	1	–	**Ovine feces**
				35	**K-S-Sf-Sxt-C**	–	–	1	**Human**
					Sf	–	–	2	**Human**
					N	–	–	1	**Human**
					Susceptible	–	–	1	Human
	Caracas	**2**	**1521**	**1**	Susceptible	–	1	–	Ovine feces
				2	**Sf**	–	1	–	**Ovine feces**
	Poona	**2**	**812**	**18**	Susceptible	–	2	–	Ovine feces
	Enteritidis	**3**	**11**	**4**	Susceptible	–	–	1	Human
				5	Susceptible	–	–	1	Human
				6	Susceptible	–	1	–	Ovine feces
	Typhi	**2**	**1**	**23**	**Sf**	–	–	1	**Human**
				26	**Sf**	–	–	1	**Human**
	Paratyphi B	**32**	**86**	**15**	**Ak-K-S-Sf-Sxt-C**	–	–	1	**Human**
					Fox-Kf-Sf	–	–	1	**Human**
					Fox-Sf	–	–	1	**Human**
					S-Sf	–	–	2	**Human**
					Sf	–	–	16	**Human**
					Susceptible	–	–	7	Human
				16	**Sf**	–	–	2	**Human**
					Susceptible	–	–	2	Human
diarizonae		**3**	[ND]	**30**	Susceptible	–	3	–	Ovine feces
Total number of isolates		**161**				**59**	**52**	**50**	

[1] ST does not exist since *dna*N gene could not be amplified.
[ND] Phenotypically antimicrobial resistant isolates are indicated in bold character.

3.2. Serotyping with low prediction accuracy

Although serotyping is the most commonly used subtyping method for *Salmonella*, it is well known that serotyping has several drawbacks, with low amount of data, high cost, and a necessity for great skill (Franklin et al., 2011; McQuiston et al., 2004). Serotyping is a labor-intensive method, and the possibility always exists for false positive results. And in the current study, it was observed that serotyping was misleading for 14 isolates (8.7%) among 161 positive isolates (Table 2). For example, the isolates representing the serotypes, *S.* Sandiego, *S.* Othmarschen, *S.* Sandiego and *Salmonella* subsp. *salamae* were selected to be re-serotyped three times due to unmatching results with MLST as well as PFGE results. The final decision of serotyping was given as a result of all molecular subtyping methods (Table 2). At the end, it was observed that serotyping had given a prediction accuracy rate of 93%, which is comparable with literature (Zou et al., 2012). Since false-positive reactions may take place due to weak, nonspecific agglutination during serotyping (Schrader et al., 2008), these false results suggest that molecular detection methods are essential for control and confirmation of traditional methods.

3.3. Four novel STs found in our study

MLST studies revealed 10, 15, and 6 Sequence Types (STs) in food, animal and clinical human isolates, respectively (Table 1). And MLST discrimination power was different for different hosts. For example for food and clinical human *S.* isolates, SID of MLST ($SID_{food,MLST} = 0.832$, $SID_{human,MLST} = 0.567$) was almost same as serotyping, while for animal fecal isolates MLST provided higher discrimination ($SID_{animal,MLST} = 0.890 > SID_{animal,serotype} = 0.821$).

A total of four new sequence types (ST 1807, ST 1822, ST 1831, ST 1832 representing *S.* Kentucky, *S.* Newport, *S.* Reading, and *S.* Othmarschen serotypes, respectively) were newly assigned in to the databank of the UoW Achtman research group. The sequence information are now publicly available in UoW website. The phylogenetic trees were developed based on concatenated 7 genes for each source group (Fig. 1).

Among the food isolates, the sequence types; *S.* Othmarschen (ST 1832) and *S.* Montevideo (ST 195); *S.* Infantis (ST 32) and *S.* Typhimurium (ST 19); and lastly *S.* Newport (ST 166) and *S.* Hadar (ST 33); were clustered on close branches in the phylogenetic tree (Fig. 1). Although the number of *S.* Newport isolates was few (n = 4), three different STs (ST 31, ST 166, and ST 1822) were observed (Table 1), which indicates a possibility of a great diversity specific to this serotype.

3.4. PFGE diversified isolates with same serotypes and ST groups

Pulsed field gel electrophoresis (PFGE), representing the best discrimination power, distinguished 18 PFGE patterns (PTs) in food isolates, where animal isolates showed 27 PTs and human clinical isolates were represented by 10 PTs. For 161 *S.* isolates 43 indistinguishable PFGE patterns with SID value of 0.945 were observed, showing that discrimination power of PFGE was higher than MLST and serotyping in this current study ($SID_{food,PFGE} = 0.897$, $SID_{animal,PFGE} = 0.956$, $SID_{human,PFGE} = 0.682$).

Certain serotypes and STs were diversified into more than one PFGE patterns. For example the serotypes, *S.* Montevideo, *S.* Telaviv and *S.* Enteritidis were the most diverged serotypes found by PFGE. Nine, six, and three PFGE patterns were represented by these serotypes, respectively (Table 1).

Although, most of the serotypes represented by two, or more than two, isolates were diverted into more than one PFGE pattern, there were some exceptional clonal isolates such as; *S.* Anatum (n = 12), *S.* Hadar (n = 2), and *S.* Poona (n = 2). For example, isolates representing *S.* Anatum (n = 12) shared only one ST and PT (Table 1). Identical PFGE patterns for these isolates may be explained by the persistence of this serotype over food animals or there are large suppliers of these mammals to retail markets in Sanliurfa region causing the dissemination of this pattern.

PFGE patterns for clinical human and animal or food isolates were divergent in common with some exceptions. When isolate subgroups were analyzed (Table 1), PT 8 was found to be specific to serotype *S.* Infantis associated with chicken meats (i.e., 80% of the Infantis isolates had that featured PT 8). Thus, it was concluded that PT 8 was significantly associated with chicken and chicken meats.

PFGE as well as MLST showed that human isolates were more clonal than isolates from other sources in this study. The most common PFGE pattern among clinical human isolates was PT 15 (n = 28) with the serotype, *S.* Paratyphi B. This might be due to the high number of *S.* Paratyphi B isolates as well as the host-restricted nature of *S.* Paratyphi B (Prager et al., 2003).

A total of two PFGE patterns (PT 10 and PT 13) were observed in all three different host species. The common isolated serotypes were *S.* Kentucky (n = 14) and *S.* Typhimurium (n = 11); and they were grouped into 2 and 3 PTs correspondingly. While, PT 10, representing *S.* Kentucky, was detected in two different food samples (1 ground beef meat, 1

Table 2
PFGE and MLST as a control and confirmation for serotypes.

No	Strain ID	First serotyping result	Estimated serotype by PFGE (PT)	Estimated serotype by MLST (ST)	Second serotyping result	Decision on serotype
1	MET-S1-087	Othmarschen	Similar to Montevideo (PT 27) [1]	Othmarschen (Novel ST:1832)	Othmarschen	Othmarschen
2	MET-S1-103	Virchow	Infantis (PT 8)	Infantis (ST 32)	Infantis	Infantis
3	MET-S1-204	Paratyphi B	Typhimurium (PT 35)	Typhimurium (ST 19)	Typhimurium	Typhimurium
4	MET-S1-227	Othmarschen	similar to Paratyphi B (PT 29) [2]	Othmarschen (Novel ST:1832)	Othmarschen	Othmarschen
5	MET-S1-324	subsp. *salamae*	Montevideo (PT 22)	Montevideo (ST 195)	–	Montevideo
6	MET-S1-396	subsp. *salamae*	Montevideo (PT 25)	Montevideo (ST 195)	–	Montevideo
7	MET-S1-401	Sandiego	Telaviv (PT 38)	Telaviv (ST 1068)	–	Telaviv
8	MET-S1-402	Paratyphi B	Telaviv (PT 38)	Telaviv (ST 1068)	Chester	Telaviv
9	MET-S1-416	Reading	Reading (PT 17)	Reading (ST 93)	Chester	Reading
10	MET-S1-439	subsp. *salamae*	Montevideo (PT 19)	Montevideo (ST 195)	–	Montevideo
11	MET-S1-512	Enteritidis	Montevideo (PT 22)	Montevideo (ST 195)	–	Montevideo
12	MET-S1-625	Newport	Typhimurium (PT 13)	Typhimurium (ST 19)	Typhimurium	Typhimurium
13	MET-S1-704	Saintpaul	Reading (PT 17)	Reading (ST 93)	Chester	Reading
14	MET-S1-709	subsp. *salamae*	Montevideo (PT 19)	Montevideo (ST 195)	Montevideo	Montevideo

[1] PFGE pattern of MET-S1–087 clustered with *S.* Montevideo isolates.
[2] PFGE pattern of MET-S1–087 clustered with *S.* Paratyphi B isolates.

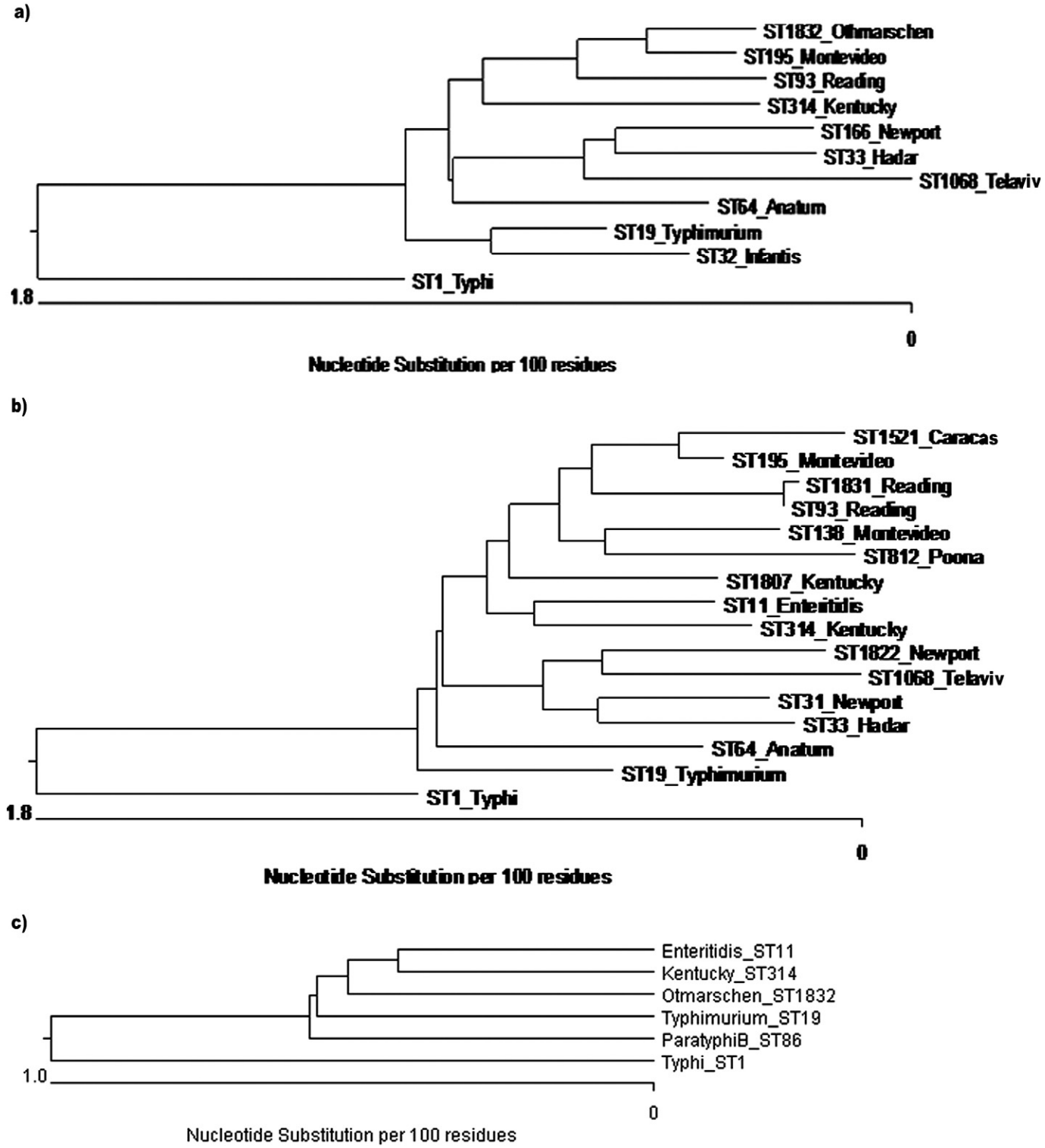

Fig. 1. Phylogenetic trees of *Salmonella* isolates according to their concatenated sequences of 7 housekeeping genes in *S. enterica* MLST scheme. Sequences of 7 genes from one representative of each a) food, b) animal, c) human *Salmonella* isolates were concatenated, and a 3336-base–pair-sequence was formed for each subtype. Concatenation was conducted with following order: *aroC* (501 nt), *dnaN* (501 nt), *hemD* (432 nt), *hisD* (501 nt), *purE* (399 nt), *sucA* (501 nt), *thrA* (501 nt). In order to avoid negative branch lengths in the figure, cladogramic view of neighbor-joining tree was selected. Phylogenetic tree of the detected STs were rooted by ST 1 of serotype Typhi as the out-group.

offal), bovine samples (1 bovine fecal) and 5 clinical human isolates; PT 13, representing *S.* Typhimurium, was observed in food (1 offal), 2 different animal samples (1 bovine fecal, 1 ovine fecal) and 2 clinical human isolates (Fig. 2). Interestingly, *S.* Telaviv, the possible emerging serotype in Turkey (Durul et al., 2015), had dissociated into 6 different PTs; which was high compared to most of the other serotypes.

3.5. Different AR profiles for each source group; food, animal and clinical human isolates

Phenotypic antimicrobial susceptibility profile test results can be interpreted according to the source of isolate. In food isolates, *S.* Infantis had attracted attention, since all of the *S.* Infantis isolates had shown

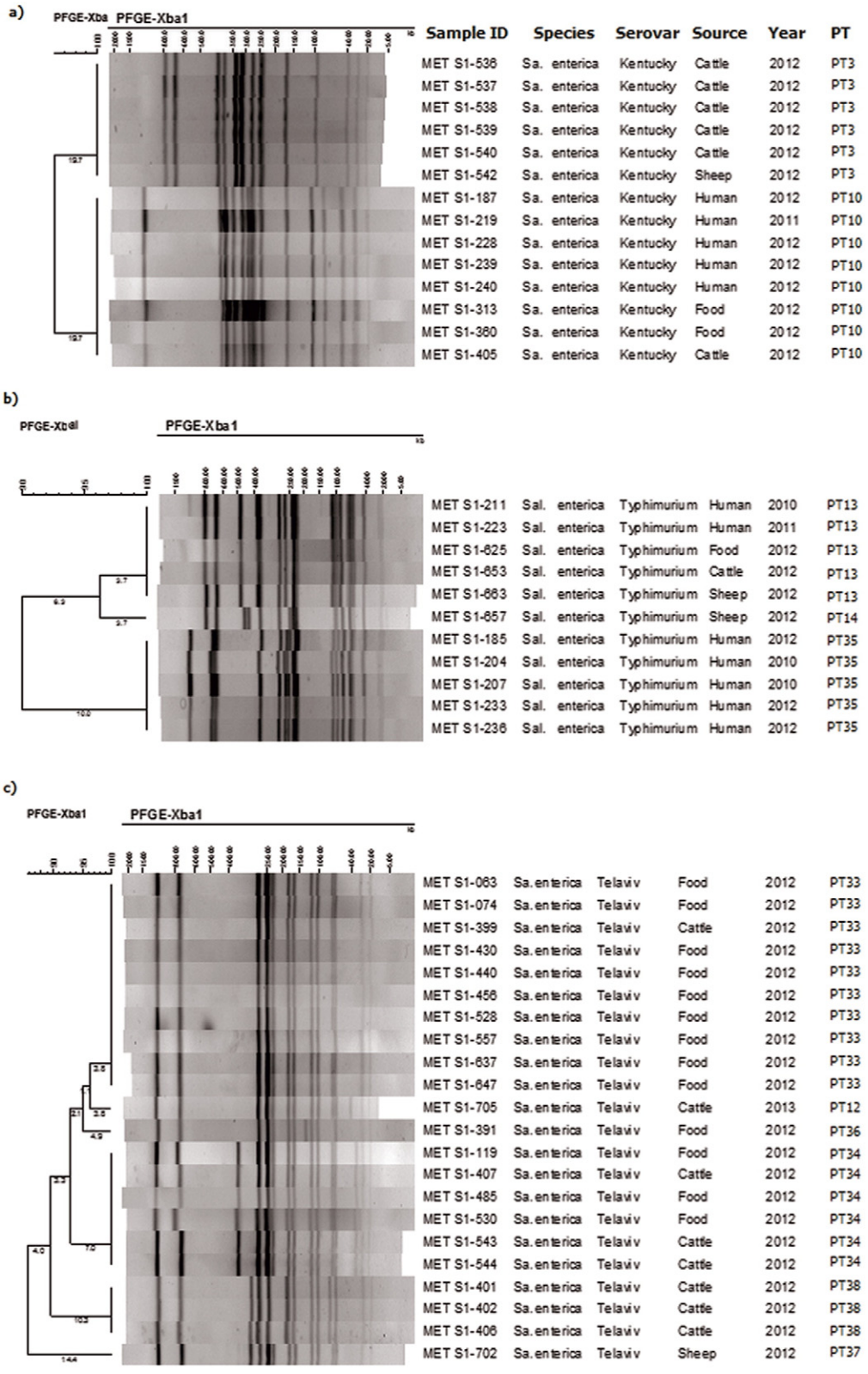

Fig. 2. Dendograms for *Salmonella* isolates by the restriction fragments created by *Xba*I enzyme. a) *S.* Kentucky, b) *S.* Typhimurium, c) *S.* Telaviv. Similarities were determined by dice coefficient and the patterns were compared by UPGMA (unweighted pair group method with arithmetic mean). Settings of 1.5% band position tolerance and 1.5% optimization was used.

resistance to at least to two antimicrobial agents. Every *S.* Infantis isolate was resistant to nalidixic acid and tetracycline; and nearly all of them were resistant to streptomycin and sulfonamide. These results agreed with the findings of other studies performed in Germany (Miko et al., 2005) and the U.S. (White et al., 2001).

The diversity of AR (resistant to at least one antimicrobial) profiles of animal-related *Salmonella* isolates was different than the food-related and human-related isolates. The beta lactams did not have the same impact on the animal-origin isolates compared to food-origin isolates. Fox-Kf-Ert (cefoxitin, cephalothin and ertapenem) resistance was observed

Table 3
Genotypic and phenotypic correlation found in resistant strains for given antimicrobial groups.

		Food isolates		Animal isolates		Clinical human isolates		Total isolates	
Antimicrobial group	Type	Number of AR isolates	Kappa[1] value	Number of AR isolates	Kappa value	Number of AR isolates	Kappa value	Number of AR isolates	Kappa value
Aminoglycoside	Genotype[2]	22	**0.93**	4	0.73	2	0.79	28	**0.90**
	Phenotype	23		6		3		32	
β-lactam	Genotype	5	**1.00**	6	0.67	4	**1.00**	15	**0.89**
	Phenotype	5		9		4		18	
Tetracycline	Genotype	23	**1.00**	2	0.49	1	0.65	26	**0.90**
	Phenotype	23		5		2		30	
Sulfonamide	Genotype	21	0.44	1	0.11	3	0.00	25	0.14
	Phenotype	30		9		36		75	
Trimethoprim	Genotype	0	0.00	0	0.00	0	0.00	0	0.00
	Phenotype	2		0		2		4	
Chloramphenicol	Genotype	1	**1.00**	0	0.00	0	0.00	1	0.39
	Phenotype	1		1		2		4	

[1] The Cohen's Kappa statistic is a measure of the agreement above that expected by chance, a kappa of 0 indicates that there is no agreement and a value of 1 indicates a complete agreement. Strong agreement between groups (>0.80) was indicated in bold character.
[2] The resistance phenotype was to streptomycin, kanamycin or amikacin, and the resistance genotype was *aadA1/2, strA/B, or aphA$_{1\text{-}IAB}$*.

in one *S.* Montevideo isolated from bovine. Fox-Kf (cefoxitin and cephalothin) resistance was seen in four serotypes; *S.* Montevideo, *S.* Hadar, and *S.* Paratyphi B. The food-origin *S.* Hadar serotype had shared the same AR with animal-origin one; streptomycin, nalidixic acid, ampicillin, tetracycline and cephalothin resistance (S-N-Amp-T-Kf). In addition to these groups of antimicrobials, in animal-origin one, additionally amoxicillin-clavualic acid, cefoxitin, and ertapenem resistance were also observed. All of the *S.* Typhimurium isolates (3/3) had shown resistance to ampicillin and tetracycline. On the other hand, the serotypes; *S.* Enteritidis, *S.* Poona and *Salmonella* subsp. *diarizonae* were susceptible to 18 different antimicrobial agents. In human isolates, on the other hand, sulfonamide resistance was very common and observed in 75% of isolates.

3.6. Geographical, as well as host, clusters of AR genes

A total of 72 (21 food, 15 animal and 35 human) phenotypically resistant *Salmonella* isolates were screened for the presence of 21 AR genes to determine the responsible genes for phenotypes in isolates from different hosts. Firstly, kappa statistics were measured to assess the agreement between phenotypic and genotypic data within each antimicrobial group (Table 3). The presence of genes (*aadA1*, *aadA2*, *strA*, *strB*, *aphA$_{1\text{-}IAB}$*, *bla$_{TEM\text{-}1}$*, *bla$_{PSE\text{-}1}$*, *tetA*) have shown very good correlation with the resistance phenotypes to aminoglycoside, beta-lactam, and tetracyclines (kappa ≥ 0.8). This result indicated that the majority of genes that encoded the resistance phenotype had been included in our study. However, for chloramphenicols and sulfonamides resistances, poor correlation (kappa ≤0.4) was observed between phenotypic and genotypic data (Table 3). Miscorrelation of phenotypic and molecular methods in human cases (especially for *S.* Paratyphi B) might be due to the antimicrobial genes that were chosen for nontyphoidal *Salmonella* isolates.

The AR genes in this study were selected based on their confirmed phenotypic-associations (Soyer et al., 2013), prevalence in literature and presence in the lists of National Antimicrobial Resistance Monitoring System (NARMS). But there might be geographical differences among genotypic AR profiles among different countries. For example a study in U.S. in 2004 (Soyer et al., 2013) revealed that 50% of human and bovine-origin *Salmonella* isolates, which were resistant to aminoglycosides and beta-lactams, carried *bla$_{CMY\text{-}2}$* or *ampC*, but in our study we did not find any isolates harboring these genes. Also most of the aminoglycoside resistance had been related with *strA* and *strB* genes in the U.S. study (Soyer et al., 2013), the findings from our study, again, did not agree with this study. Furthermore, in a different study (Chen et al., 2004), AR profiles of *Salmonella* isolates obtained from retail meats in U.S. and China had shown that the resistance profiles change territorially. Whereas U.S. isolates had *bla$_{CMY\text{-}2}$* gene for resistance to beta-lactamase group of antimicrobial drugs, *bla$_{TEM\text{-}1}$* gene was present in the isolates obtained from Chinese products. Thus, the genotypic AR profiles in this study might have been specific to its geography and therefore this may have been the reason of not detecting some common AR genes in our isolates.

Table 4
The distribution of antimicrobial resistance genes associated with phenotypic serotypes detected in *Salmonella* isolates.

		Serotypes (number of isolates)		
Antimicrobial agent group	Genes screened	Food isolates	Animal isolates	Clinical human isolates
Aminoglycoside	*aadA1*	*S.* Infantis (14)	ND	ND
	aadA2	ND	*S.* Typhimurium (1)	ND
	strA	*S.* Infantis (3)	ND	ND
	strB	*S.* Hadar (1)	*S.* Hadar (1), *S.* Typhimurium (2)	ND
	aphA$_{1\text{-}iab}$	*S.* Infantis (9)	ND	*S.* Paratyphi B (1)
Tetracycline	*tetA*	*S.* Infantis (15), *S.* Hadar (1), *S.* Typhimurium (1)	*S.* Hadar (1), Typhimurium (1)	*S.* Typhimurium (1)
Beta-lactam	*bla$_{TEM\text{-}1}$*	*S.* Infantis (2), *S.* Hadar (1), *S.* Typhimurium (1)	*S.* Montevideo (1), *S.* Hadar (1), *S.* Typhimurium (2)	*S.* Typhimurium (2), *S.* Paratyphi B (2)
	bla$_{PSE\text{-}13}$	ND	*S.* Typhimurium (1)	ND
Sulfonamide	*sul1*	*S.* Infantis (14)	*S.* Typhimurium (1)	Kentucky (2), Typhi (1)
Phenicol	*cmlA*	*S.* Infantis (1)	ND	ND

ND: Not detected.

Among 24 streptomycin resistant food isolates, 14 of them (58%) had the *aadA1* gene and none of the isolates with streptomycin resistance carried *aadA2* or *aacC2* genes. But, for animal isolates, different than food-origin isolates, no *aadA1* gene was detected; conversely *aadA2* gene was detected in one isolate (*S.* Typhimurium) that was obtained from sheep (Table 4). The *strB* gene was only detected from two *S.* Hadar isolates. Strong association (100%) was observed between $aphA_{1\text{-}iab}$ gene presence and kanamycin resistance. Tetracycline resistance was related with the *tetA* gene in all *Salmonella* isolates. Beta-lactam resistance in food-origin *Salmonella* isolates was related with only $bla_{TEM\text{-}1}$ gene (Table 4).

Although beta-lactam resistance had a wide spectrum in animal-origin *Salmonella* isolates compared to other sources, according to the molecular detection results, only two beta-lactam resistance genes ($bla_{TEM\text{-}1}$ and $bla_{PS13E\text{-}1}$) were detected among them. Here, it was concluded that the prevalence of AR genes might have been related with geography and also the source and serotype of the isolates.

Nearly half of the MDR isolates (15/34) were representing *S.* Infantis, which were collected from chicken samples (n = 15), and it highlighted that a great effort should be taken to investigate the reasons of contamination in chicken farms and consequences of this case.

Serotype associated AR genes were studied by potential unconditional statistical associations among the seven serotypes (*S.* Infantis, *S.* Typhimurium, *S.* Hadar, *S.* Paratyphi B, *S.* Kentucky, *S.* Typhi and *S.* Montevideo). The odds of identifying *aadA1*, *tetA*, $aphA_{1\text{-}IAB}$, *sul1*, genes in *S.* Infantis were 7.4, 5.7, 4.8 and 3.7 times higher (95% CI) than *Salmonella* non-Infantis isolates (Table 5). The unconditional association found between the resistance genes in chicken meat origin *Salmonella* Infantis isolates proposed that there might be a possibility of mobile genetic elements transfer through these isolates since there is a co-selection of resistance to the same classes of antimicrobials.

Similar to our study, researchers demonstrated that there was an emergence of *S.* Infantis in Israel (Aviv et al., 2014; Gal-Mor et al., 2010), which had been associated with a megaplasmid found on the emerging isolates. Furthermore, the antimicrobial resistance profiles of broiler chickens in Hungary (Nógrády et al., 2007) harboring MDR *S.* Infantis clones were similar to that of our isolates; and it has been reported that the possibility of spread of these isolates to individuals through chicken meat may result in a significant threat to public health.

4. Conclusion

Characterization of *Salmonella* isolates collected from animal and human, as well as foods in Sanliurfa region provided a better understanding of transmission (i.e. transmission of *Salmonella* to humans) and ecology of *Salmonella* in that region. From our knowledge, this study is the first study in Turkey that analyzes the phenotypic features of *Salmonella* isolates by genetic methods through the farm to fork chain. Antimicrobial resistance had differed according to source of isolate. For example, the aminoglycoside resistance was predominant in food isolates, however beta-lactam resistance was higher in animal isolates. Occurrence of different AR gene profiles designated a potential association of isolates between source, serotype and geography. The reason for not observing a possible local serotype, *S.* Telaviv and persistent and MDR *S.* Infantis, in human isolates may be related with its low virulence capacity and limited sample size. On the other hand, a rare serotype, *S.* Othmarschen, was collected from both food and human sources. Presence of such serotypes, especially MDR ones, has potential to cause severe cases in humans in the future, and it underlines the importance of food safety. Our work entitles the sequence types and PFGE patterns possible endemic to Turkey and submits the diversity of *Salmonella* in this region by subtyping and antimicrobial susceptibility methods. By establishing a web-based databank (foodmicrobetracker.com; Pathogen Detector: pathogendetector-metu.rhcloud.com) it was ensured to build a permanent and solid *Salmonella* archive for the future studies in Turkey.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijfoodmicro.2016.09.031.

Table 5
Association of antimicrobial resistance genes recovered from phenotypically resistant food, animal and human isolates.

Outcome gene	Predictor serotype	Log odds ratio [1]	95% CI	*p*-value
aadA1	*S.* Infantis	7.39	54.57–48173.43	$p < 0.0001$
tetA	*S.* Infantis	5.71	15.83–5787.99	0.0001
$aphA_{1\text{-}IAB}$	*S.* Infantis	4.77	12.45–1118.69	0.0001
sul1	*S.* Infantis	3.65	8.34–177.66	$p < 0.0001$

[1] The statistically significant unconditional associations from a logistic regression model are listed (*p*-value of 0.05/20 comparisons; $p < 0.0025$).

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References

Achtman, M., Wain, J., Weill, F.X., Nair, S., Zhou, Z., Sangal, V., Krauland, M.G., Hale, J.L., Harbottle, H., Uesbeck, A., Dougan, G., Harrison, L.H., Brisse, S., Group, S.E.M.S., 2012. Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. PLoS Pathog. 8, e1002776.

Altman, D.G., 1990. Practical Statistics for Medical Research. CRC Press.

Aviv, G., Tsyba, K., Steck, N., Salmon-Divon, M., Cornelius, A., Rahav, G., Grassl, G.A., Gal-Mor, O., 2014. A unique megaplasmid contributes to stress tolerance and pathogenicity of an emergent *Salmonella enterica* serovar Infantis strain. Environ. Microbiol. 16, 977–994.

Barrett, T.J., Gerner-Smidt, P., Swaminathan, B., 2006. Interpretation of pulsed-field gel electrophoresis patterns in foodborne disease investigations and surveillance. Foodborne Pathog. Dis. 3, 20–31.

Boxrud, D., Pederson-Gulrud, K., Wotton, J., Medus, C., Lyszkowicz, E., Besser, J., Bartkus, J.M., 2007. Comparison of multiple-locus variable-number tandem repeat analysis, pulsed-field gel electrophoresis, and phage typing for subtype analysis of *Salmonella enterica* serotype Enteritidis. J. Clin. Microbiol. 45 (2), 536–543.

Carletta, J., 1996. Assessing agreement on classification tasks: the kappa statistic. Comput. Linguist. 22, 249–254.

Chen, S., Zhao, S., White, D.G., Schroeder, C.M., Lu, R., Yang, H., McDermott, P.F., Ayers, S., Meng, J., 2004. Characterization of multiple-antimicrobial-resistant *Salmonella* serovars isolated from retail meats. Appl. Environ. Microbiol. 70, 1–7.

CLSI, 2012. CLSI Performance Standards for Antimicrobial Susceptibility Testing; 22nd Informational Supplement, M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA.

Davis, M., Morishita, T.Y., 2005. Relative ammonia concentrations, dust concentrations, and presence of *Salmonella* species and *Escherichia coli* inside and outside commercial layer facilities. Avian Dis. 49, 30–35.

Dohoo, I.R., Martin, S.W., Stryhn, H., 2009. Veterinary Epidemiologic Research. VER, Incorporated.

Durul, B., Acar, S., Bulut, E., Kyere, E.O., Soyer, Y., 2015. Subtyping of *Salmonella* food isolates suggests the geographic clustering of serotype Telaviv. Foodborne Pathog Dis. 12, 958–965.

ECDC, 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA J. 13, 3991.

Erol, I., 1999. Ankara'da tüketime sunulan kıymalarda *Salmonella* ların varlığı ve serotip dağılımı. Turk. J. Vet. Anim. Sci. 23, 321–325.

EUCAST, 2012. Antimicrobial Susceptibility Testing. EUCAST Disk Diffusion Method. The European Committee on Antimicrobial Susceptibility Testing.

Ferris, M.M., Yan, X., Habbersett, R.C., Shou, Y., Lemanski, C.L., Jett, J.H., Yoshida, T.M., Marrone, B.L., 2004. Performance assessment of DNA fragment sizing by high-sensitivity flow cytometry and pulsed-field gel electrophoresis. J. Clin. Microbiol. 42, 1965–1976.

Foley, S.L., Lynne, A.M., 2008. Food animal-associated *Salmonella* challenges: pathogenicity and antimicrobial resistance. J. Anim. Sci. 86, E173–E187.

Franklin, K., Lingohr, E.J., Yoshida, C., Anjum, M., Bodrossy, L., Clark, C.G., Kropinski, A.M., Karmali, M.A., 2011. A rapid geno-serotyping tool for the classification of *Salmonella* serovars. J. Clin. Microbiol. (02347–02310).

Gal-Mor, O., Valinsky, L., Weinberger, M., Guy, S., Jaffe, J., Schorr, Y.I., Raisfeld, A., Agmon, V., Nissan, I., 2010. Multidrug-resistant *Salmonella enterica* serovar Infantis, Israel. Emerg. Infect. Dis. 16, 1754.

Girard, M.P., Steele, D., Chaignat, C.L., Kieny, M.P., 2006. A review of vaccine research and development: human enteric infections. Vaccine 24, 2732–2750.
Grimont, P., Weil, F., 2007. Antigenic Formulae of the *Salmonella* Servovars. 9th ed. Pasteur Institute, Paris (World Health Organization Centre for Reference and Research on *Salmonella*).
Hawker, J., Begg, N., Blair, I., Reintjes, R., Weinberg, J., Ekdahl, K., 2012. Communicable Disease Control and Health Protection Handbook. John Wiley & Sons.
Herikstad, H., Motarjemi, Y., Tauxe, R.V., 2002. *Salmonella* surveillance: a global survey of public health serotyping. Epidemiol. Infect. 129, 1–8.
Kim, J.S., Lee, G.G., Park, J.S., Jung, Y.H., Kwak, H.S., Kim, S.B., Nam, Y.S., Kwon, S.T., 2007. 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*. J. Food Prot. 70, 1656–1662.
Lee, L.A., Puhr, N.D., Maloney, E.K., Bean, N.H., Tauxe, R.V., 1994. Increase in antimicrobial-resistant *Salmonella* infections in the United States, 1989–1990. J. Infect. Dis. 170, 128–134.
Maiden, M.C., 2006. Multilocus sequence typing of bacteria. Annu. Rev. Microbiol. 60, 561–588.
Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A., Hoekstra, R.M., 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin. Infect. Dis. 50, 882–889.
McQuiston, J., Parrenas, R., Ortiz-Rivera, M., Gheesling, L., Brenner, F., Fields, P., 2004. Sequencing and comparative analysis of flagellin genes *fliC*, *fljB*, and *flpA* from *Salmonella*. J. Clin. Microbiol. 42, 1923–1932.
Miko, A., Pries, K., Schroeter, A., Helmuth, R., 2005. Molecular mechanisms of resistance in multidrug-resistant serovars of *Salmonella enterica* isolated from foods in Germany. J. Antimicrob. Chemother. 56, 1025–1033.
Murase, T., Nagato, M., Shirota, K., Katoh, H., Otsuki, K., 2004. Pulsed-field gel electrophoresis-based subtyping of DNA degradation-sensitive *Salmonella enterica* subsp *enterica* serovar Livingstone and serovar Cerro isolates obtained from a chicken layer farm. Vet. Microbiol. 99, 139–143.
Nógrády, N., Tóth, Á., Kostyák, Á., Pászti, J., Nagy, B., 2007. Emergence of multidrug-resistant clones of *Salmonella* Infantis in broiler chickens and humans in Hungary. J. Antimicrob. Chemother. 60, 645–648.
Ochiai, R.L., Wang, X., von Seidlein, L., Yang, J., Bhutta, Z.A., Bhattacharya, S.K., Agtini, M., Deen, J.L., Wain, J., Kim, D.R., Ali, M., Acosta, C.J., Jodar, L., Clemens, J.D., 2005. *Salmonella* Paratyphi A rates, Asia. Emerg. Infect. Dis. 11, 1764–1766.
Prager, R., Rabsch, W., Streckel, W., Voigt, W., Tietze, E., Tschäpe, H., 2003. Molecular properties of *Salmonella enterica* serotype Paratyphi B distinguish between its systemic and its enteric pathovars. J. Clin. Microbiol. 41, 4270–4278.
Ribot, E.M., Fair, M.A., Gautom, R., Cameron, D.N., Hunter, S.B., Swaminathan, B., Barrett, T.J., 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog. Dis. 3, 59–67.
Richardson, A., 1975. Outbreaks of bovine salmonellosis caused by serotypes other than *S.* Dublin and *S.* Typhimurium. J. Hyg. (Lond.) 74, 195–203.
Schrader, K.N., Fernandez-Castro, A., Cheung, W.K., Crandall, C.M., Abbott, S.L., 2008. Evaluation of commercial antisera for *Salmonella* serotyping. J. Clin. Microbiol. 46, 685–688.
Soyer, Y., Richards, J., Hoelzer, K., Warnick, L., Fortes, E., McDonough, P., Dumas, N., Gröhn, Y., Wiedmann, M., 2013. Antimicrobial drug resistance patterns among cattle-and human-associated *Salmonella* strains. J. Food Prot. 76, 1676–1688 (®).
Swaminathan, B., Barrett, T.J., Hunter, S.B., Tauxe, R.V., Force, C.D.C.P.T., 2001. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg. Infect. Dis. 7, 382–389.
Teh, C.S., Chua, K.H., Thong, K.L., 2014. Paratyphoid fever: splicing the global analyses. Int. J. Med. Sci. 11, 732–741.
White, D.G., Zhao, S., Sudler, R., Ayers, S., Friedman, S., Chen, S., McDermott, P.F., McDermott, S., Wagner, D.D., Meng, J., 2001. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. N. Engl. J. Med. 345, 1147–1154.
Zou, W., Lin, W.-J., Hise, K.B., Chen, H.-C., Keys, C., Chen, J.J., 2012. Prediction system for rapid identification of *Salmonella* serotypes based on pulsed-field gel electrophoresis fingerprints. J. Clin. Microbiol. 50, 1524–1532.