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A molecular epidemiological investigation of multistate outbreaks of *Salmonella Enteritidis* from clinical and environmental samples in Turkey, 2000–2010

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Background/aim: To analyze interrelated *Salmonella* Enteritidis strains isolated in Turkey. Fifty-five S. Enteritidis surveillance strains were isolated from human feces and environmental samples from different regions in Turkey between 2000 and 2010.

Materials and methods: Clinical isolates were selected from different outbreaks in the Turkish National Reference Laboratory. All isolates were submitted to antimicrobial susceptibility test, plasmid profile analysis, and Xba*I*-digested pulsed-field gel electrophoresis.

Results: The strains were scanned against 20 antibiotics and for 3 of them (amikacin, ciprofloxacin, gentamicin), all strains were found to be sensitive. Five isolates had no plasmid. Most of test strains carried the 57-kb plasmid in common and 15 genotypes were identified among the 55 isolates. Six genotypes were related closely, 3 genotypes were undistinguished, and 6 genotypes were unrelated.

Conclusion: To our knowledge, this is the first report on the phenotypic and molecular characterization of *S.* Enteritidis isolates from both environmental samples and clinical isolates in Turkey.

Key words: *Salmonella* Enteritidis, plasmid, pulsed-field gel electrophoresis, antimicrobial sensitivity

1. Introduction

Salmonellosis is a common and major health problem worldwide. It has been identified as a consequential zoonotic pathogen in animals and humans (1). In 2006, 121 *Salmonella* outbreaks took place in the United States, causing more than 3300 cases reported to the Centers for Disease Control and Prevention (CDC) foodborne outbreak reporting system (2). *Salmonella* causes estimated millions of illnesses and hundreds of deaths annually in many countries (3). According to the CDC, in 2009 and 2010, a total of 1527 foodborne disease outbreaks (675 in 2009 and 852 in 2010) were reported and *Salmonella* was the second highest cause, accounting for 30% of outbreaks (4). It is estimated that *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S.* Enteritidis) causes more than 200,000 cases of illness annually (2,5). *S.* Enteritidis is one of the most common serotypes of *Salmonella* bacteria reported worldwide (5). It has been the primary cause of *Salmonella* outbreaks in many countries. Before 1980, *Salmonella* serotype Typhimurium was more commonly isolated than *S.* Enteritidis, but since the 1980s there has been considerable increase in the number of reported findings of this serotype, not only in developing countries but also in developed ones (6). The major sources of *S.* Enteritidis outbreaks have been identified as poultry and poultry products since the early 2000s. International travel and contact with reptiles have also been associated with *S.* Enteritidis infection (5). *S.* Enteritidis isolates have been characterized with phenotyping and genotyping methods in outbreak research (3). Nevertheless, phenotypic methods are not very effective for epidemiological analysis of *Salmonella* transmission because they cannot discriminate accurately between isolates that are closely related (7).

Nowadays genotyping methods are proposed for typing bacteria (3), having been developed for high-power genetic discrimination of *S.* Enteritidis isolates during outbreaks (1). A number of molecular typing techniques such as ribotyping, restriction fragment length polymorphism, plasmid profile analysis, multilocus variable number of tandem repeat analysis (8,9), and DNA microarray analysis (10) have been used to identify *S.* Enteritidis isolates in outbreaks.

Pulsed-field gel electrophoresis (PFGE) is currently known as the gold standard for subtyping of many bacteria worldwide (2). It is used routinely by the CDC and in state

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health departments in Latin America, Asia, and the United States (1). The efficacy of PFGE regarding discrimination and epidemiological characterization of *S.* Enteritidis strains has been proven (2).

To our knowledge, this is the first study on antimicrobial resistance and molecular characterization of *S.* Enteritidis from environmental samples sourced from poultry farms and humans in Turkey. The purpose of this study was to determine and compare antimicrobial resistance, plasmid profiling, and PFGE patterns of related *S.* Enteritidis strains isolated from different geographical area during the period from 2000 to 2010. The present study is an attempt to help the surveillance of antimicrobial resistance status between 2000 and 2010 in Turkey.

2. Materials and methods

2.1. Bacterial strains

A total of 55 *S.* Enteritidis strains were studied. These strains were isolated from human feces (46) and environmental samples (9) from 9 different cities in Turkey from 2000 through 2010. These strains were selected from the collection of the Public Health Institution of Turkey, National Reference Laboratory for Enteric Pathogens. Isolates were systematically chosen to represent isolates from outbreaks and environmental samples that occurred during different years. Table 1 lists the year and the isolation source of the 55 *S.* Enteritidis strains used in this study.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibilities for *S.* Enteritidis isolates were determined by the standard disk diffusion method in Mueller-Hinton agar in accordance with Clinical and Laboratory Standards Institute guidelines. All strains were tested for resistance to the following 20 antibiotics (Oxoid, UK): ampicillin (AMP) (10 μg), gentamicin (GN) (10 μg), amoxicillin-clavulanic acid (AMC) (25 μg), cefuroxime sodium (CXM) (30 μg), cefoperazone (CFP) (30 μg), cefotaxime (CTX) (30 μg), ceftizoxime (ZOX) (30 μg), ceftriaxone (CRO) (30 μg), ceftazidime (CAZ) (30 μg), sulfamethoxazole/trimethoprim (SXT) (25 μg), chloramphenicol (C) (30 μg), tetracycline (TE) (10 μg), kanamycin (K) (30 μg), nalidixic acid (NA) (30 μg), ciprofloxacin (CIP) (5 μg), sulfonamides (S3) (30 μg), streptomycin (S10) (10 μg), trimethoprim (W) (25 μg), cefpodoxime (CPD) (10 μg), and amikacin (AK) (30 μg). *E. coli* ATCC 25922 was used as the quality control strain.

Table 1. Plasmid types and molecular sizes of all isolates.

Strains	Plasmid patterns (kb)	Plasmid types	Number of strains	Percentage of strains
2, 3, 12, 14, 15, 17, 19, 20, 28, 18, 34, 41, 42, 46, 32	57; 3, 7	Type 1	15	27.20%
4, 7, 8, 9, 22, 25, 43, 44, 49	57; 5	Type 2	9	16.3%
5	67; 3,7	Type 3	1	1.81%
6, 13, 14	57; 3,7; 3,4	Type 4	1	5.4%
10	67; 5; 3,7; 3,4	Type 5	1	1.81%
11, 40, 53, 55, 51, 54	57	Type 6	6	10.9%
29	57; 5; 4	Type 7	1	1.81%
35, 39, 38	57; 1, 5	Type 8	3	5.4%
45	57; 3, 4	Type 9	1	1.81%
23	90; 57; 9; 3, 4	Type 10	1	1.81%
16	67; 7	Type 11	1	1.81%
37	3, 7	Type 12	1	1.81%
36	67; 57	Type 13	1	1.81%
24	67; 1, 5	Type 14	1	1.81%
26	67; 57; 4; 3, 4	Type 15	1	1.81%
31	67	Type 16	1	1.81%
33	67; 5	Type 17	1	1.81%
30	67; 3, 4	Type 18	1	1.81%
21	2, 8	Type 19	1	1.81%
1, 27, 48, 50, 52	No plasmid	Type 20	5	9.09%
	Total		55	

2.3. Plasmid DNA analysis

Plasmid DNA of each strain was extracted and purified according to the method of Kado and Liu (10) with modifications and determined by electrophoresis on 0.6% agarose gel (Sigma-Aldrich, USA) containing 0.5 mL of ethidium bromide with 1X Tris-borate-EDTA (TBE) buffer at 110 V for 3.5 h. A large molecular marker, supercoiled DNA ladder (Invitrogen, Carlsbad, CA), and *E. coli* 239 (147 kb, 63 kb, 36 kb) were used for determining plasmid size. Control strains were acquired from the Public Health Institution of Turkey, National Reference Laboratory for Enteric Pathogens.

2.4. Pulsed field gel electrophoresis

Agarose blocks were prepared according to the CDC Pulse Net protocol with some modifications (11). They were digested with the restriction endonuclease *Xba*I (Fermentas Life Sciences, Lithuania) overnight in a water bath at 37 °C. Fragments were separated by electrophoresis for 19.4 h at 6 V/cm with pulse times of 2.2–63.8 s and at 14 °C in 0.5X TBE buffer, and were electrophoresed with a CHEF-DRII electrophoresis system (Bio-Rad, USA). The gels were stained with ethidium bromide (2 mg/mL, Sigma) for 25 min and then rinsed 3 times with distilled water for 15 min each and visualized with a UV transilluminator. Photographed images were converted to TIFF files. The band patterns were analyzed by BioNumerics (Applied Maths, Inc., Belgium) software version 6.01. Cluster analysis was obtained at 1% optimization and 1% tolerance, and DNA relatedness was calculated based on the Dice coefficient and unweighted pair group method with averages (UPGMA). *Salmonella* Braenderup (H9812) was used as a molecular weight marker for normalization. The DNA banding patterns were interpreted as instructed by Tenover et al. (12,13).

3. Results

3.1. Antimicrobial susceptibility test

The antimicrobial susceptibility of 55 *S.* Enteritidis strains isolated from human and environmental samples were determined. Table 2 summarizes the resistance of all *S.* Enteritidis strains to 21 antimicrobial agents. Among the strains, the highest levels of resistance were observed for cefotaxime and ceftizoxime (12.7%); cefpodoxime and ceftazidime (10.9%); sulfonamides and ampicillin (5.45%); cefuroxime sodium, chloramphenicol, and nalidixic acid (3.63%); and trimethoprim, streptomycin, sulfamethoxazole-trimethoprim, ceftriaxone, and cefoperazone (1.8%). Some of the isolates were found to have lower resistance to cefuroxime sodium (10.9%); tetracycline (5.45%); streptomycin, nalidixic acid, ceftriaxone, and ampicillin (3.6%); and cefoperazone, amoxicillin-clavulanic acid, ceftazidime, and kanamycin (1.8%), but no strains were resistant to gentamicin, ciprofloxacin, or amikacin.

In this study, multiresistance, the resistance to 2 or more antimicrobials, occurred in 16 (29%) of the isolates, and it seems that multiresistance in human *S.* Enteritidis isolates is a problem for public health.

In Turkey epidemiological data were observed identifying antimicrobial resistance of *S.* Enteritidis in 9 different cities, and all strains from Erzurum, Malatya, Denizli, and Alanya were found susceptible to all drugs. The highest level of resistance among the cities was found in Kütahya and Ankara.

3.2. Plasmid DNA analysis

A total of 20 different plasmid profiles were observed among the 55 *S.* Enteritidis isolates. They were identified by their weight in kilobase pairs (kb). Fifty isolates had 1 to 6 plasmids with molecular sizes from 1.5 to 90 kb. Type 6 carried a single plasmid of approximately 57 kb (72.7%). Ten distinct plasmid types carried this major plasmid, along with others, while 9.1% of isolates were plasmid-free *S.* Enteritidis strains. Table 2 shows plasmid types and molecular sizes for all isolates. Tested strains having different antibiotic resistance patterns carried similar plasmid profiles or their direct opposites. The data showed that there was not a close relation between antibiotic resistance and plasmids. It could be due to single genetic event with a deletion, insertion, or point mutation on the plasmids of isolates.

3.3. PFGE analysis

After the *S.* Enteritidis strains were examined by PFGE method and cut with an XbaI macrorestriction enzyme, a band ranging between 11 and 16 was obtained. Thirty-eight clinical isolates belonged to 3 pulsotypes (types 1, 2, and 3) and had a similarity coefficient higher than 95%. They were classified a clonally related to each other. Eleven clinical isolates belonged to 6 pulsotypes (3A, 4, 4A, 5, 5A, and 5B), and they were highly genetically homogeneous and had more than 85% similarity. Six isolates belonged to 6 pulsotypes (types 6, 7, 8, 9, 10, and 11) and had unrelated profiles. In addition, type 1 was found to be the most prevalent in the environmental samples. These results emphasized that ongoing transmission was very common from human to human and from human to environment. The Figure shows the results obtained by PFGE for *S.* Enteritidis isolates and the phylogenetic tree of all isolates. Recently, infections with type 1 of *S.* Enteritidis have been predominant in Turkey.

Therefore, all strains that belonged to the dominant *S.* Enteritidis profile (type 1) were cut with a second macrorestriction enzyme (*Spe*I) for confirmation. All type 1 *S.* Enteritidis strains were confirmed as being the same as each other.

4. Discussion

Salmonella infections are a global problem; the serotyping of *Salmonella* strains posing a danger for public health

Table 2. Numbers of all *Salmonella* Enteritidis isolates with pulsed field gel electrophoresis (PFGE) type, antimicrobial resistance profile, plasmid profile, origin, sources and dates.

Strain no.	Source	Origin	Date	PFGE type	Plasmid type	Resistance to antibiotics
1	Feces	Düzce	2000	1	20	Sensitive to all
2	Feces	Düzce	2007	3	1	CTX, ZOX, CAZ
3	Feces	Erzurum	2004	1	1	Sensitive to all
4	Feces	Malatya	2002	1	2	Sensitive to all
5	Feces	Kütahya	2002	1	3	CFP, C, W
6	Feces	Ankara	2004	7	4	KF, ZOX, SXT
7	Feces	Ankara	2009	1	2	CAZ, CPD
8	Feces	Ankara	2009	1	2	CTX, CAZ, CPD
9	Feces	Ankara	2005	3	2	Sensitive to all
10	Feces	Ankara	2005	8	5	Sensitive to all
11	Feces	Ankara	2009	1	6	AMP, CXM, CTX, ZOX, TE
12	Feces	Ankara	2009	1	1	Sensitive to all
13	Feces	Ankara	2009	1	4	CAZ
14	Env. sample	Ankara	2009	1	4	Sensitive to all
15	Feces	Erzurum	2009	1	1	Sensitive to all
16	Env. sample	Ankara	2010	6	11	CIP, CPD
17	Feces	Erzurum	2009	1	1	Sensitive to all
18	Feces	Diyarbakır	2009	1	1	S3
19	Feces	Diyarbakır	2009	1	1	Sensitive to all
20	Feces	Erzurum	2009	1	1	Sensitive to all
21	Feces	Ankara	2009	1	19	Sensitive to all
22	Feces	Ankara	2005	10	2	S3, CPD
23	Feces	Malatya	2001	1	10	Sensitive to all
24	Feces	Alanya	2009	1	14	Sensitive to all
25	Feces	Ankara	2003	2	2	CRO
26	Feces	Ankara	2009	9	15	Sensitive to all
27	Feces	Ankara	2004	11	20	Sensitive to all
28	Feces	Alanya	2005	2	1	Sensitive to all
29	Feces	Diyarbakır	2009	1	7	Sensitive to all
30	Feces	Ankara	2004	10	18	C, CPD
31	Feces	Ankara	2005	4A	16	AMP, CTX, ZOX
32	Feces	Ankara	2009	1	1	Sensitive to all
33	Env. sample	Ankara	2009	1	17	CPD
34	Env. sample	Ankara	2009	1	1	Sensitive to all
35	Feces	Manisa	2009	1	8	Sensitive to all
36	Env. sample	Ankara	2009	1	13	C
37	Env. sample	Ankara	2009	1	12	Sensitive to all
38	Feces	Manisa	2009	1	8	CXM, CTX, ZOX
39	Feces	Manisa	2009	1	8	Sensitive to all
40	Feces	Denizli	2009	5A	6	Sensitive to all
41	Feces	Manisa	2009	1	1	Sensitive to all

Table 2. (Continued).

42	Env. sample	Ankara	2009	1	1	Sensitive to all
43	Feces	Kütahya	2009	1	2	K
44	Feces	Manisa	2009	1	2	CAZ
45	Feces	Kütahya	2009	5	9	S3, S10, CPD
46	Env. sample	Ankara	2009	1	1	CAZ
47	Feces	Diyarbakır	2009	1	7	CTX, ZOX, CAZ, CPD
48	Feces	Manisa	2009	5A	20	Sensitive to all
49	Env. sample	Ankara	2010	5A	2	CTX, ZOX
50	Feces	Ankara	2005	4	20	Sensitive to all
51	Feces	Ankara	2010	5A	6	AMP, CTX, ZOX
52	Feces	Manisa	2009	5A	20	CTX, ZOX
53	Feces	Denizli	2009	5B	6	Sensitive to all
54	Feces	Ankara	2002	1	6	Sensitive to all
55	Feces	Denizli	2009	5A	6	Sensitive to all

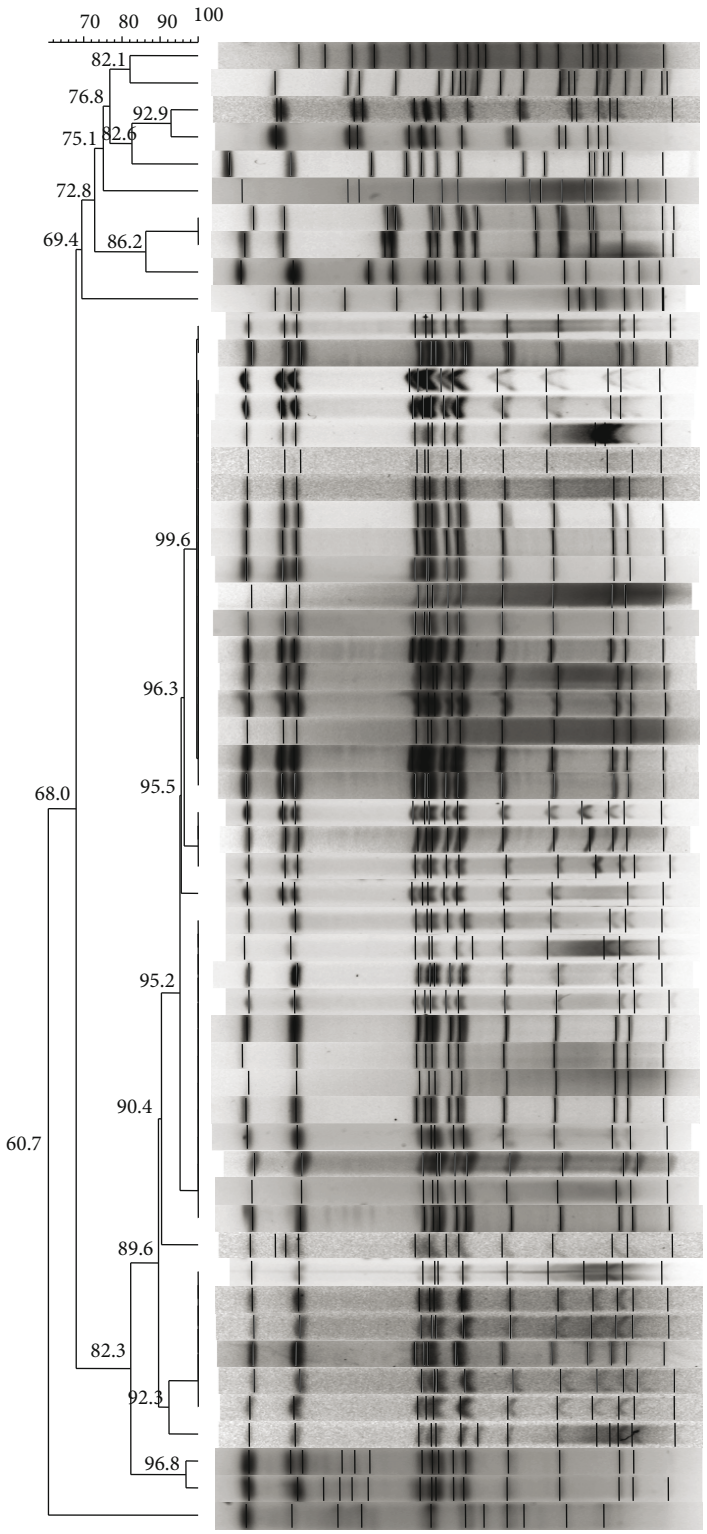
and the determination of antimicrobial sensitivities are of importance epidemiologically (14). *S.* Enteritidis is the second most common serovar seen in South America and Oceania (15–18). It is the most common serovar seen in other areas, including Turkey (19). It was reported by the National Enteric Pathogens Reference Laboratory of Turkey that 47% of human-based *Salmonella* strains in 2000–2002, 46% of human-based *Salmonella* strains in 2003–2005, and 79.4% of *Salmonella* strains isolated from clinical samples and 26% of *Salmonella* strains obtained from nonclinical isolates in 2010 were *S.* Enteritidis (20).

In this study, 55 *S.* Enteritidis strains isolated from environmental and clinical samples were typed by means of plasmid DNA profile and PFGE methods. Antimicrobial resistance patterns of samples were examined in this study, conducted over a long period and throughout Turkey.

Antibiotic resistance, which is increasing rapidly in *Salmonella* species, is an important public health problem. Castro et al. (21) examined 128 clinical *S.* Enteritidis isolates between 1985 and 1999 and found that 0.8% were resistant to nalidixic acid and 8.6% were resistant to sulfamethoxazole/trimethoprim. Fernandes et al. (6) found that 20.9% of 105 isolates were resistant to nalidixic acid and 13.9% were resistant to sulfamethoxazole/trimethoprim. In Turkey, Erdem et al. (22) determined high resistance to chloramphenicol, ampicillin, and sulfamethoxazole/trimethoprim antibiotics used in *Salmonella* treatment. Between 2003 and 2005, it was reported that 46% of 87 clinical and nonclinical *Salmonella* isolates were *S.* Enteritidis, 5.7% of which showed resistance to nalidixic acid (20,23), and it was observed that nonclinical *S.* Enteritidis isolates were resistant to gentamicin, streptomycin, nalidixic acid, sulfamethoxazole/trimethoprim, and ampicillin. This study covered the years between 2000 and 2010 years and an increase was observed in clinical isolate resistance profile in Turkey compared with previous years: 12% ceftizoxime, 12% ceftriaxone, and 10% ceftazidime resistance profiles were observed. Similarity was found in the antibiotic resistance profiles of environmental and clinical samples. Environmental isolates in this study came to the National Reference Enteric Laboratory for confirmation and typing from poultry farms, and resistances in these strains may have resulted from excess antibiotic usage for the purpose of increasing growth, particularly in local production. Antibiotics used in animals may lead to the development of resistant pathogens infecting humans through the food chain. For this reason, the use of antimicrobial agents in humans and animals should be done with caution.

Plasmid profile analysis is a fast, simple, and cheap molecular method used for the classification of epidemics (22,24). However, in this study, it was determined that while certain strains do not carry plasmids, the majority of strains carry plasmids specific to the *S.* Enteritidis serotype (57 kb), either singularly or with other plasmids. Since many strains have similar plasmid profiles in Turkey, it was concluded that plasmid profiles should be used together with another molecular method in the monitoring of *S.* Enteritidis epidemiology.

Further separation of epidemic strains is required, and methods based on DNA fingerprint analysis have been frequently used in recent years (22). PFGE is used in the subtyping of the *S.* Enteritidis serotype and is accepted as the gold standard among molecular methods (25). In a prior study, repetitive extragenic palindromic based-PCR, enterobacterial repetitive intergenic consensus sequence based-PCR, and Box-PCR were used together in typing *S.* Enteritidis strains, but it was reported that this



Stratin No	PFGE Tipi	Places	Date	Sources
St 16	6	ES ANKARA	2010	Env. Samp.
St 6	7	ANKARA	2004	FECES
St 50	4	ANKARA	2005	FECES
St 31	4A	ANKARA	2005	FECES
St 10	8	ANKARA	2005	FECES
St 26	9	ANKARA	2009	FECES
St 2	3	DÜZCE	2007	FECES
St 9	3	ANKARA	2005	FECES
St 22	3A	ANKARA	2005	FECES
St 30	10	ANKARA	2004	FECES
St 34	1	ESANKARA	2009	Env.Samp.
St 13	1	ANKARA	2009	FECES
St 41	1	MANİSA	2009	FECES
St 42	1	ES ANKARA	2009	Env.Samp.
St 46	1	ES ANKARA	2009	Env.Samp.
St 33	1	ES ANKARA	2009	Env.Samp.
St 44	1	MANİSA	2009	FECES
St 1	1	DÜZCE	2000	FECES
St 3	1	ERZURUM	2004	FECES
St 32	1	ANKARA	2009	FECES
St 24	1	ALANYA	2009	FECES
St 29	1	DİYARBAKIR	2009	FECES
St 47	1	DİYARBAKIR	2009	FECES
St 12	1	ANKARA	2009	FECES
St 15	1	ERZURUM	2009	FECES
St 17	1	ERZURUM	2009	FECES
St 19	1	DİYARBAKIR	2009	FECES
St 18	1	DİYARBAKIR	2009	FOOD .
St 14	1	ES ANKARA	2009	Env.Samp.
St 21	1	ANKARA	2009	FECES
St 36	1	ESANKARA	2009	Env.Samp.
St 37	1	ES ANKARA	2009	Env.Samp.
St 43	1	KÜTAHYA	2009	FECES
St 39	1	MANİSA	2009	FECES
St 38	1	MANİSA	2009	FECES
St 35	1	MANİSA	2009	FECES
St 54	1	ANKARA	2002	FECES
St 8	1	ANKARA	2009	FECES
St 4	1	MALATYA	2002	FECES
St 7	1	ANKARA	2009	FECES
St 23	1	MALATYA	2001	FECES
St 11	1	ANKARA	2009	FECES
ST 20	1	ERZURUM	2009	FECES
St 5	1	KÜTAHYA	2002	FECES
St 45	5	KÜTAHYA	2009	FECES
St 40	5A	DENİZLİ	2009	FECES
St 48	5A	MANİSA	2009	FECES
St 52	5A	MANİSA	2009	FECES
St 55	5A	DENİZLİ	2009	FECES
St 51	5A	ANKARA	2010	FECES
St 49	5A	ES ANKARA	2010	Env.Saples
St 53	5B	DENİZLİ	2009	FECES
St 28	2	ALANYA	2005	FECES
St25	2	ANKARA	2003	FECES
ST 27	11	ANKARA	2004	FECES

Figure. Dendrogram generated using the Dice coefficient based on PFGE profiles of the 55 *Salmonella* Enteritidis isolates restricted with XbaI, constructed using UPGMA.

did not find an effective difference for *S.* Enteritidis (15). Soyer et al. (26) tried the PFGE and multilocus sequence typing methods together and Ridley et al. (9) tried random amplified polymorphic DNA and ribotyping, and they reported that PFGE had the highest discrimination power.

Strains carrying similar antibiotic resistance profiles were identified with the PFGE method and divided into types. In our study, we showed that the same resistance profile may belong to different clones. When antibiotic resistance models are examined together with plasmid profiles and PFGE models, it is observed that strains have different characters from each other. This study revealed that when PFGE is used with antibiogram and plasmid profiles, it may be beneficial for both revealing the genetic relationship among strains having different resistances and the separation of strains showing the same resistance phenotype.

Surveillance studies related with foodborne *Salmonella* epidemics have only been established in Turkey recently, and for this reason there is no adequate documentation yet. Important studies by Aktaş et al. (27), carried out on *S.* Enteritidis strains isolated from clinical isolates, typed 26 *S.* Enteritidis strains isolated from pediatric units with PFGE and plasmid DNA profile analysis methods, and Us et al. (13) typed *S.* Enteritidis strains isolated from different provinces with the PFGE method. Kilic et al. (2) carried out an evaluation of a foodborne epidemic in Isparta with the PFGE method. In the study of *S.* Enteritidis typing carried out by Us et al. (13) in Turkey, the dominant PFGE model obtained following cutting the strains with the *Xba*I enzyme differed from the PFGE model obtained from clinical and nonclinical isolates in this study, while the *S.* Enteritidis profile found by Kilic et al. (2) was consistent with the dominant profile in this study. There are limited numbers of study carried out on *S.* Enteritidis isolated from environmental samples (23), and studies on typing are inadequate.

This is the first study on the molecular characterization of *S.* Enteritidis from both environmental samples and clinical isolates in Turkey, and this is a multicenter study covering 9 provinces, so it provides an understanding of the molecular epidemiologic structure and antimicrobial resistance of *S.* Enteritidis in Turkey.

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References

1. Pate M, Micunovic J, Bole-Hribovsek V, Lah AK, Raunik M, Kosir M, Harlander M, Cretnik TJ. Investigation of two *Salmonella* serovar Enteritidis outbreaks using the pulsed-field gel electrophoresis: a good example of collaboration at the national level. Slov Vet Res 2011;48: 99–105.
2. Kilic A, Bedir O, Kocak N, Levent B, Eyigun CP, Tekbas OF, Gorenek L, Baylan O, Basustaoglu AC. Analysis of an outbreak of *Salmonella* Enteritidis by repetitive-sequence based PCR and pulsed-field gel electrophoresis. Inter Med 2010; 49: 31–36.
3. Ammari S, Laglaoui A, En-nanei L, Bertrand S, Wildemauwe C, Barrijal S, Abid M. Characterization of *Salmonella* Enteritidis isolated from foods and patients in northern Morocco. J Infect Dev Ctries 2009; 3: 695–703.
4. CDC. Surveillance for Foodborne Disease Outbreaks — United States, 2009–2010. MMWR 2013; 62: 41–47.
5. CDC. Surveillance for Foodborne Disease Outbreaks — United States, 2006. Atlanta, GA, USA: CDC; 2009.
6. Fernandes SA, Ghilardi AC, Tavechio AT, Machado AM, Pignatari AC. Phenotypic and molecular characterization of *Salmonell*a Enteritidis strains isolated in Sao Paulo, Brazil. Rev Inst Med Trop Sao Paulo 2003; 45: 59–63.
7. Cho S, Whittam TS, Boxrud DJ, Bartkus JM, Saeed AM. Allele distribution and genetic diversity of VNTR locin *Salmonella enterica* serotype Enteritidis isolates from different sources. BMC Microbiol 2008; 8: 146–157.
8. Zou W, Lin WJ, Foley SL, Chen SH, Nayak R, Chen JJ. Evaluation of pulsed field gel electrophoresis profiles for ıdentification of *Salmonella* serotypes. J Clin Microbiol 2010; 48: 3122–3126.
9. Ridley AM, Threlfall EJ, Rowe B. Genotypic characterization of *Salmonella enteritidis* phage types by plasmid analysis, ribotyping and pulsed-field gel electrophoresis. J Clin Microbiol 1998; 36: 2314–2321.
10. Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 1981; 145: 1365–73.
11. CDC. PulseNet Pathogens and Protocols. Atlanta, GA, USA: CDC; 2013.
12. Tenover EC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; 33: 2233–2239.
13. Us E, Erdem B, Tekeli A, Gerçeker D, Saran B, Bayramova M, Şahin F. Investigation of *Salmonella* serotype Enteritidis isolates by plasmid profile analysis and pulsed field gel electrophoresis. Mikrobiyol Bul 2011; 45: 210–227 (in Turkish with English abstract).
14. Ghozzi-Abbassi I, Jaouani A, Aissa RB, Martinez-Urtaza J, Boudabous A, Gtari M. Antimicrobial resistance and molecular analysis of non-typhoidal *Salmonella* isolates from human in Tunisia. Pathologie Biologie 2011; 59: 207–212.

15. Campioni F, Bergamini AM, Falcao JP. Genetic diversity, virulence genes and antimicrobial resistance of *Salmonella* Enteritidis isolates from food and humans over a 24-year period in Brazil. Food Microbiol 2012; 32: 254–264.

16. Humphrey T. *Salmonella* stress responses and food safety. Nat Rev Microbiol 2004; 2: 504–509.

17. Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Humphrey TJ, Van Immerseel F. Mechanisms of egg contamination by *Salmonella enteritidis*. FEMS Microbiol Rev 2009; 33: 718–738.

18. Hendriksen RS, Vieira AR, Karlsmose S. Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. Foodborne Pathog Dis 2011; 8: 887–900.

19. Ben Aissa R, Al-Gallas N, Troudi H, Belhadj N, Belhadj A. Trends in *Salmonella enterica* serotypes isolated from human, food, animal, and environment in Tunisia, 1994-2004. J Infect 2007; 55: 324–339.

20. Levent B, Sezen F, Güleşen R, Gözalan A, UEPLA Çalışma Grubu. Ulusal Enterik Patojenler Laboratuvar Sürveyans Ağı (UEPLA) *Salmonella* ve *Shigella* suşları ve antimikrobiyal direnç durumları, 2007-2009. In: XXXIV. Türk Mikrobiyoloji Kongresi, Antalya, Turkey; 2006. p. 234 (in Turkish).

21. Castro FA, Dos Santos VR, Gomes Martins CH, Fernandes SA, Zaia JE, Martinez R. Prevalence and antimicrobial susceptibility of *Salmonella* serotypes in patients from Ribeirao Preto, Sao Paulo, Brazil, between 1985 and 1999. Braz J Inf Dis 2002; 6: 244–251.

22. Erdem B, Hasçelik G, Gedikoğlu S, Gür D, Ercis S, Sümerkan B, Aysev AD, Tuncer İ, Tuğrul M, Tatman Otkun M et al. *Salmonella* enterica serotypes and *Salmonella* infections: a multicenter study covering ten provinces in Turkey. Mikrobiyol Bul 2004; 38: 173–186 (in Turkish with English abstract).

23. Kalender H, Şen S. Analysis of *Salmonella enterica* subsp. *enterica* serovar Enteritidis strains isolated from chicken and chicken meat by pulsed field gel electrophoresis and phage typing. İstanbul Üniversitesi Veteriner Fakültesi Dergisi 2008; 34: 15–24 (in Turkish with English abstract).

24. Helmuth R, Stephan R, Bunge C, Hoog B, Steinbeck A, Bulling E. Epidemiology of virulence associated plasmids and outer membrane protein patterns with seven common *Salmonella* serovars. Infect Immun 1985; 48: 175–182.

25. Oliveira SD, Bessa MC, Santos LR, Itapema Cardoso MRI, Brandelli A, Canal CW. Phenotypic and genotypic characterization of *Salmonella* Enteritidis isolates. B J Microbiol 2007; 38: 720–728.

26. Soyer Y, Alcaine SD, Schoonmaker-Bopp DJ, Root TP, Marnick LD, McDonough PL, Dumas NB, Gröhn YT, Wiedmann M, Schoonmaker-Bopp DJ. Pulsed-field gel electrophoresis diversity of human and bovine clinical Salmonella isolates. Foodborne Pathog Dis 2010; 7: 707–717.

27. Aktas Z, Day M, Kayacan CB, Diren S, Threlfall EJ. Molecular characterization of *Salmonella* Typhimurium and *Salmonella* Enteritidis by plasmid analysis and pulsed-field gel electrophoresis. Int J Antimicrob Agents 2007; 30: 541–545.