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Genotyping results of *Salmonella* Infantis as a food poisoning agent in Turkey between 2013 and 2017

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Abstract: The aim of this study is to define the genotyping relationship between 31 *Salmonella enterica* subspecies *enterica* serovar Infantis (*S.* Infantis) strains, which were the most isolated and identified causes of food poisoning between 2013 and 2017 in Turkey. These isolated strains were studied with the DiversiLab System (repetitive sequence-based PCR; rep-PCR; bioMerieux, France) and traditional serotyping for the identification of *S.* Infantis was also carried out. Totally, 31 *S.* Infantis isolates were genotyped. DNA was extracted from each isolate using the Microbial DNA Isolation Kit as well as rep-PCR. The dendrogram shows the diversity of the observed samples contained in the library with 4 main clusters of *S.* Infantis (26 strains) being significantly different from each other with a similarity of more than 95% between strains. Furthermore, it was found that the cause of almost all of these cases originated from chicken or chicken-based products. Even though the isolated *S.* Infantis strains came from different geographical locations, after genotyping, their genetic profiles were found to be similar. This is the first retrospective study concerning the molecular characterization of *S.* Infantis isolates obtained from food poisoning cases between 2013 and 2017 in Turkey.

Key words: *Salmonella* Infantis, food, food poisoning, genotyping

1. Introduction

Salmonella, an essential food-borne pathogen, causes illness worldwide [1]. It was confirmed that *S.* Infantis was the causative agent for salmonellosis in humans in some countries. It was also the third most frequently isolated serovar of *Salmonella* after *S.* Enteritidis and *S.* Typhimurium in 2003 [2,3]. According to reports from European countries in 2014, *S.* Infantis is the dominant *Salmonella* isolate found in broiler meat (35.9%) [4]. In the same year, *S.* Infantis was the most frequently reported *Salmonella* serovar found in fowl [5]. As a result of these studies in Europe, *S.* Infantis emerged as the fourth most prevalent serovar causing human salmonellosis reported by the countries of the European Union/European Economic Area (EU/EEA) [6]. In 2015, *S.* Infantis was isolated from broilers, pigs, and humans globally [7]. In 2015 and 2016, *S.* Typhimurium and *S.* Infantis reached the same level [8]. According to the scientific reports of the European Food Safety Authority and the European Centre for Disease Prevention and Control (EFSA and ECDC) published in 2016 and 2017, in total 4786 and 5079, respectively, foodborne outbreaks (including waterborne) were reported [4,8]. In 2017, Thailand, Spain, Turkey, and India were the most commonly reported travel destinations (13.8%, 8.3%, 8.2%, and 6.7%). When compared with the prior 2 years, a higher number of samples were reported for 'meat and meat products'. *S.* Infantis was mostly associated with broiler flocks and meat with contamination rates of 46% and 51%, respectively. Some EU member states (e.g., Austria, Croatia, Hungary, Italy, Slovakia, and Slovenia) reported that most of the isolates from broilers contained *S.* Infantis. For some other member states (e.g., France and the United Kingdom), nonsubstantial numbers of isolates of *S.* Infantis were reported in broilers [8].

Molecular typing procedures like pulsed field gel electrophoresis (PFGE), rep-PCR, and multilocus sequence typing (MLST) were used effectively in *Salmonella* epidemiological and phylogenetic investigations [1]. PFGE was used for the differentiation of genetic relatedness [9]. The PCR-based genotyping procedures are expeditious, straight-forward, and more cost-effective than PFGE [10]. Compared to the reliability of both PFGE and rep-PCR methods, rep-PCR is reported to be a more preferred method due to its high discriminatory capability and precise detection of transmission links [11]. Rep-PCR was effectively used to subtype varieties of bacteria and also has a commercial semiautomated system, DiversiLab [12]. As DiversiLab for rep-PCR analysis showed increased

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discrimination compared to the procedures of PFGE, it was used to appraise the genetic resemblance of *Salmonella* strains [11,13]. Kilic et al. pointed out that the DiversiLab system may be a reasonable alternative to PFGE for the surveillance and outbreak studies of *S.* Enteritidis, since it provides a simple, rapid, and highly specific screening method that archives all gel image data [10]. The rep-PCR system was used to genetically distinguish all of the *Salmonella* isolates (except between *S.* Montevideo and *S.* London). This indicates that it can be added to the toolbox for the purpose of source tracking of foodborne pathogens associated with outbreaks [14]. In another study, rep-PCR showed the best discriminatory ability since it had the highest Simpson's index [2]. Hauser et al. [15] probed 93 epidemiologically unrelated *S.* Infantis strains detected regarding contamination along the food chain in Germany between 2005 and 2008. Molecular clonality in *S.* Enteritidis and *S.* Infantis from broilers was reported in Iran [9]. Molecular analyses (PFGE) were used to investigate the case of *S.* Infantis in Greece caused by distinct biotypes or an accomplished spreading of one clone [16]. *S.* Infantis is the most common food-associated serovar in Turkey, especially in chicken meat, and is also one of the serovars posing a risk to human health [17].

The purpose of this study is to define the genotyping relationship between *S.* Infantis strains from foods which caused poisoning between 2013 and 2017 in Turkey. These isolates are studied using the DiversiLab System (rep-PCR) and also with traditional serotyping for the identification of *S.* Infantis isolates.

2. Materials and methods

Some *S.* Infantis strains from various foods were isolated and the isolates were gathered between 2013 and 2017 in Turkey. The numbers of genotyped *S.* Infantis strains during 2013–2017 are shown in Table 1. Different geographical locations, years, strain numbers, and strain sources are listed in Tables 2a and 2b by date. The biochemical properties of *S.* Infantis isolates were investigated in accordance with standard laboratory procedures (ISO 6579:2002). Isolates were identified as *Salmonella* spp. with the VITEK 2 Compact. Serological typing was done with the Kauffmann–White schema, which genotyped a total of 31 *S.* Infantis isolates, and DNA was extracted using a DNA isolation kit (Ultraclean Microbial, Mo Bio Laboratories, Inc., QIAGEN, Carlsbad, CA, USA). In this study, the DiversiLab *Salmonella* kit was used for the rep-PCR amplification. The DiversiLab system, which involves fragment separation, used the microfluidics lab-on-a-chip technology. The 2100 Bioanalyzer (Agilent Technologies, Inc., Palo Alto, CA, USA) analysed the DNA amplicons. The examination was implemented with the web-based software (version 3.3) using the Pearson correlation coefficient in order to define the distance matrices. Moreover, the unweighted pair group method with arithmetic mean (UPGMA) was used to create dendrograms. This system automatically generates the reports, including the dendrograms, electropherograms, gel-like images, similarity matrixes, scatterplots, and selectable demographic fields, to aid in interpreting the data. In general, "different", "similar", and "indistinguishable" were defined as similarity of <95%, <97%, and >95%, respectively [18].

Table 1. Genotyped *S.* Infantis over the years between 2013 and 2017.

Year	Genotyped strain numbers
2013	5
2014	1
2015	0
2016	7
2017	18
Total	31

3. Results

It was determined that the origin of nearly all of these cases was chicken or chicken-based products. This conclusion was reached because it was detected that 28 *S.* Infantis isolates out of 31 originated from chicken or chicken-based products. The dendrogram shows the diversity of the observed samples that were contained in the library with 4 main clusters of *S.* Infantis (26 strains) being significantly different from each other with a similarity of more than 95%. The reports of the dendrogram, a similarity matrix, electropherograms, gel-like images, scatterplot, and selected demographic fields are presented in Figures 1, 2, and 3. Moreover, the dendrogram shows the diversity of the observed samples that contained 7 clusters of *S* .Infantis, of which 26 strains are nearly the same, with a similarity of more than 99% between the strains: 4 strains in 2013 (1, 2, 5, 13), 1 strain in 2014 (1), 7 strains in 2016 (5, 6, 31, 81, 125, 126, 139), and 14 strains in 2017 (2, 12, 15, 29, 39, 41, 47, 52, 62, 67, 68, 70, 105, 109) (Table 3).

4. Discussion

S. Infantis is the most commonly detected serovar in foods which causes poisoning. Some molecular studies were carried out all over the world to determine the genetic resemblance of *Salmonella* serovars isolated from various matrices, especially from chicken and chicken-based products. According to a study in Finland, *S.* Infantis was isolated the most, which was causing an increase in food

Table 2a. The number of strains and their origins (2013, 2014, 2016).

Number	Year	Strain numbers	Strain sources	City
1	2013	1	Rice with chicken	Edirne
2	2013	2	Rice with chicken	Edirne
3	2013	5	Chicken leg	Tekirdağ
4	2013	10	Chicken meat	Kayseri
5	2013	13	Fresh whole chicken	Muğla
6	2014	1	Tenderloin on plate	Rize
7	2016	5	Chicken drumstick	Ankara
8	2016	6	Marinated chicken	Tokat
9	2016	31	Chicken döner	Kayseri
10	2016	81	Raw chicken	Ankara
11	2016	125	Rice with chickpeas	Erzurum
12	2016	126	Chicken döner	Erzurum
13	2016	139	Baked chicken	Ankara

Table 2b. The number of strains and their origins (2017).

Number	Year	Strain numbers	Strain sources	City
14	2017	2	Whole chicken	Adana
15	2017	12	Whole chicken	Aydın
16	2017	15	Chicken flour	İzmir
17	2017	29	Chicken meat	Konya
18	2017	39	Whole chicken	Ankara
19	2017	41	Pasta	İzmir
20	2017	47	Chicken breast	Aydın
21	2017	51	Fresh whole chicken	Ankara
22	2017	52	Frozen chicken	Manisa
23	2017	53	Raw chicken	Aydın
24	2017	62	Whole chicken	İzmir
25	2017	63	Dessert	İzmir
26	2017	64	Salad	İzmir
27	2017	67	Tenderloin on plate	İzmir
28	2017	68	Chicken leg	İzmir
29	2017	70	Chicken meat	İzmir
30	2017	105	Frozen chicken	Urfa
31	2017	109	Chicken with potato	Tokat

poisonings [19]. In Germany, one study indicated that two significant and closely related genotypes of *S*. Infantis were transmitted from contaminated broiler meat or pork to humans, which made it a threat to human health [15]. Limited pulsed field profiles defined varieties of *S*. Enteritidis, *S*. Infantis, and *S*. Corvallis isolates gathered between 1989 and 2005 from predominantly chicken-derived origins in the Kyushu–Okinawa precincts of Japan [20]. In our study, we also detected the resemblance between the isolated *S*. Infantis serotypes from chicken and chicken products. These isolates showed quite similar molecular patterns across distinct geographical

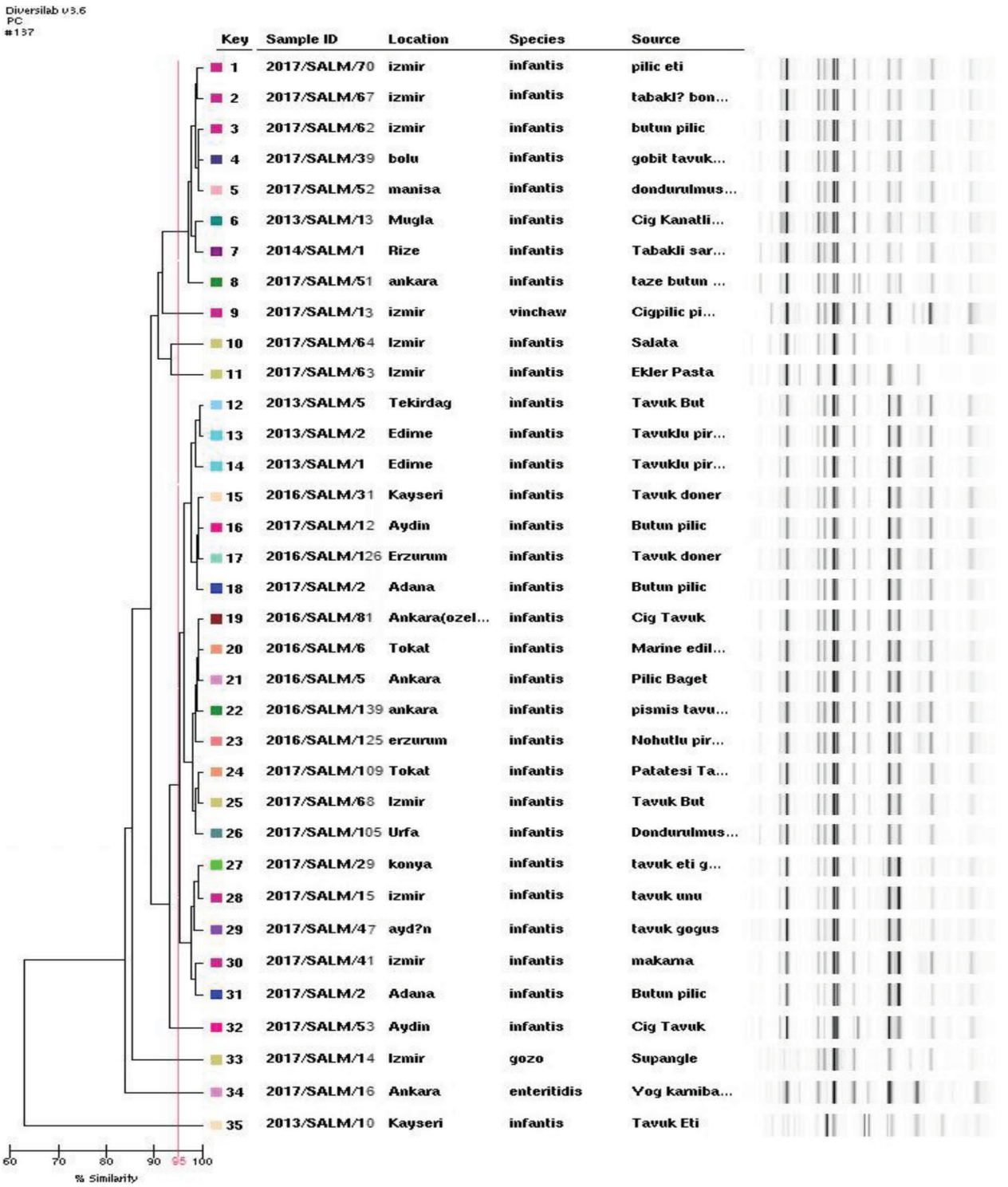

Figure 1. Dendrogram table.

locations in Iran [9]. In this study, exceedingly similar patterns indicating clonal relatedness between the *S.* Infantis strains from different geographical locations in Turkey were also found. In recent years, *S.* Infantis is overwhelmingly the serotype most consistently confronted in food-borne infections in Turkey, as is the case in the rest of the world. *S.* Enteritidis has the preponderance in humans [17]. However, according to this study, *S.* Infantis

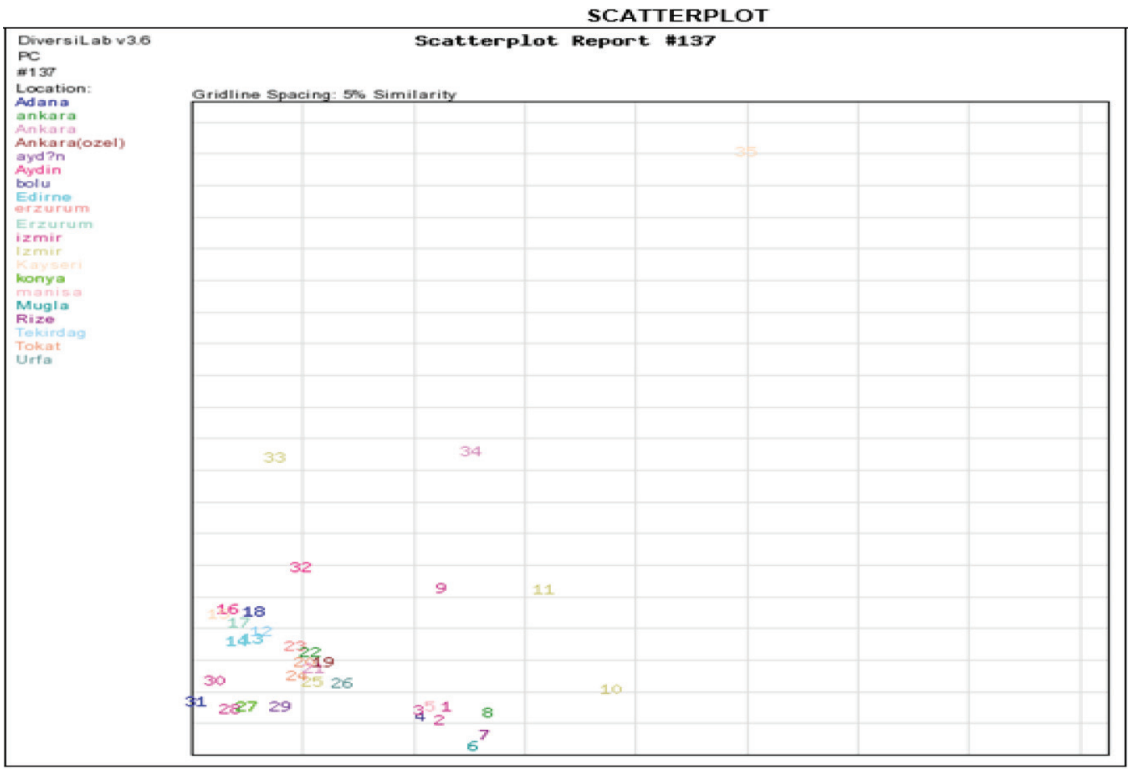

Figure 2. Scatterplot table.

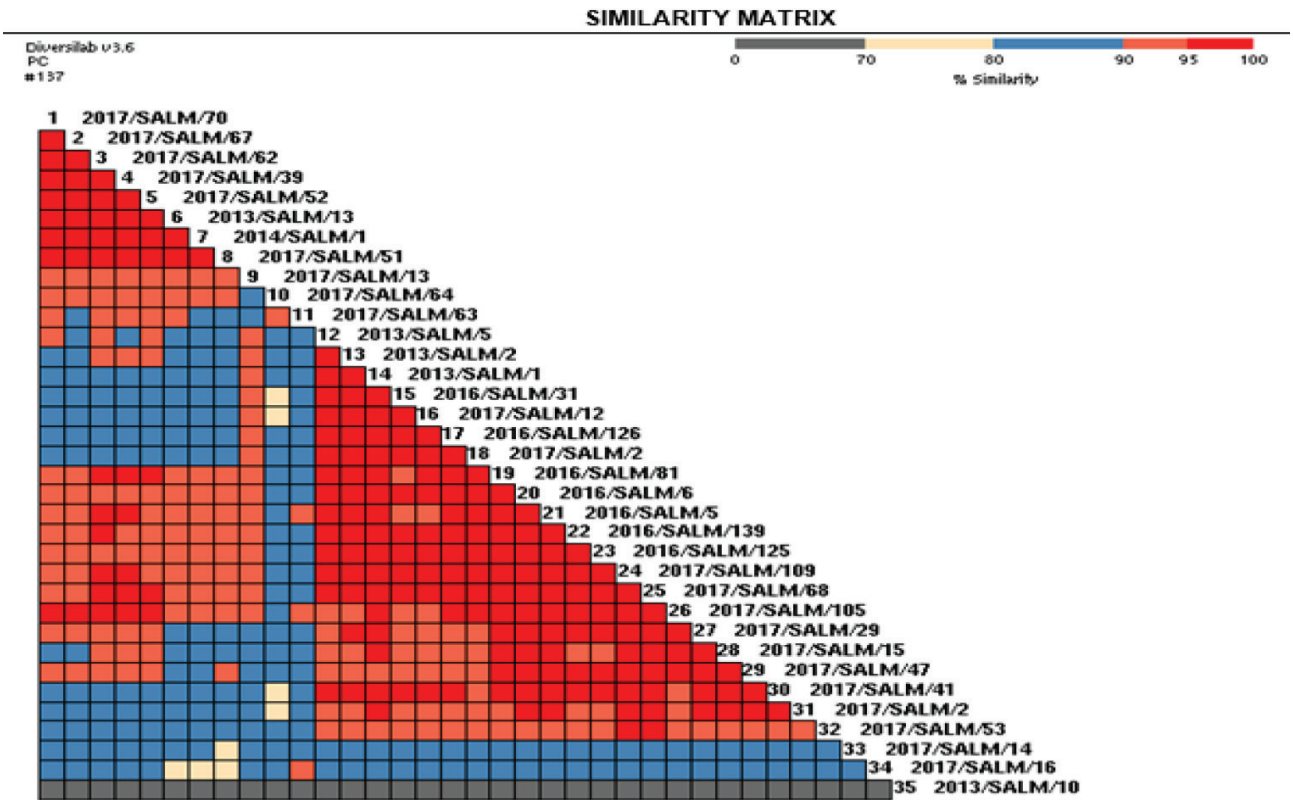

Figure 3. Interactive table.

Table 3. Seven clusters with a similarity of ≥99 %.

Year	Strain numbers and same clusters						
2013	1 (Edirne)	2 (Edirne)	5 (Tekirdağ)	13 (Muğla)			
2014	1 (Rize)						
2016	5 (Tekirdağ)	6 (Tokat)	31 (Kayseri)	81 (Ankara)	125 (Erzurum)	126 (Erzurum)	139 (Ankara)
2017	2 (Adana)	12 (Aydın)	15 (İzmir)	29 (Konya)	39 (Ankara)	41 (İzmir)	47 (Aydın)
2017	52 (Manisa)	62 (İzmir)	67 (İzmir)	68 (izmir)	70 (İzmir)	105 (Urfa)	109 (Tokat)

was the most commonly detected *Salmonella* serovar in food poisonings in Turkey between 2013 and 2017. For the first time in Turkey, an investigation of the PFGE patterns was implemented by Ozdemir and Acar in 2014 and bioinformatics procedures were used to identify both inter- and intraserotype correlations of the 4 most commonly confronted serotypes [17]. They suggested that the sources of food poisonings should be detected and genotyping investigations should be done to protect public health. In the present study, different main profiles were found among the *S.* Infantis isolates causing food poisonings. This study makes significant contributions in terms of both resources and genotypes since resources are found and the genotypes are investigated. In 2015, *S.* Infantis isolated from broiler carcasses was proven to pose a risk to human health since it was found that there was a genetic similarity of ≥92% between broiler carcasses and the infected people [5]. In Greece in 2017, PFGE showed 31 pulsotypes among 40 strains with a total similarity of 60% in the genetic makeup. That study established 4 main clusters [16]. The present study also determined 4 main clusters and detected a similarity of more than 95% between 26 strains. Us et al. [21] studied *S.* Enteritidis strains detected by PFGE and Kilic et al. (10) analysed a foodborne epidemic in Isparta, Turkey, using PFGE. Acar et al. [22] reported the first study concerning molecular relatedness of *S.* Enteritidis obtained from both environmental and clinical samples in Turkey. This study indicates a relationship between the molecular epidemiology and the antimicrobial resistance properties of *S.* Enteritidis in Turkey.

5. Conclusion

S. Infantis is the serovar most commonly detected in foods which causes poisoning. This is the first study to show the molecular relatedness of *S.* Infantis isolates obtained from food samples which caused poisoning between 2013 and 2017 in Turkey. In conclusion, this genotyping study, which was considered necessary due to the increase of *S.* Infantis-based food poisoning in Turkey (the same as in the rest of the world), revealed that the sources of the food poisonings are very similar or identical. Furthermore, it has been determined that almost all of these cases originated from chicken or chicken-based products. Even though the isolated *S.* Infantis strains originated from different geographical locations, after genotyping it was detected that the genetic profiles of the *S.* Infantis strains were similar, which indicated that the isolated *S.* Infantis strains spread from the same sources. Detecting the source of the strains by genotyping is an expedient and effective method for preserving public health.

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