



## Prevalence, Serotype Diversity and Antibiotic Resistance of *Salmonella* Among Poultry Meat and Eggs in Turkiye: A Meta-analysis

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### ABSTRACT

Poultry is a well-known reservoir for *Salmonella*, and therefore numerous outbreaks have been reported among poultry meat products and eggs. This study is aimed at determining the pooled prevalence, serotype diversity and antibiotic resistance profiles of *Salmonella* among poultry meat and eggs sold in Turkiye. For this purpose, international (Web of Science and PubMed) and national (ULAKBIM TR Index) electronic databases were searched using based on relevant keywords in English and Turkish, and out of 1,818 articles, 41 were deemed eligible for inclusion in this meta-analysis. The random effects model was accepted when a substantial heterogeneity was obtained according to Q statistics and the I<sup>2</sup> value, however the fixed effects model was assumed valid in the opposite case. The pooled prevalence of *Salmonella* in chicken parts, chicken carcasses, chicken giblets and eggs were 24.4% [95% confidence interval (CI)=17.8-32.6], 21.9% (95% CI=14.0-32.7), 20.1% (95% CI=10.7-

34.6) and 4.8% (95% CI=1.7-13.3), respectively. *Salmonella* Enteritidis was the most common serotype among eggs, chicken parts and chicken carcasses with the rates of 22.4% (95% CI=3.6-69.3), 19.0% (95% CI=3.3-61.6) and 5.8% (95% CI=2.2-14.4), respectively. The highest pooled antibiotic resistance prevalence of *Salmonella* spp., regardless of food type, was found in tetracycline (73.9%, 95% CI=51.0-88.5) (p<0.041) and ampicillin (31.5%, 95% CI=20.7-44.6). The high-pooled prevalence of the organism emphasized the potential threat *Salmonella* poses to public health, and also antibiotic resistance data revealed that the use of tetracyclines, quinolones and penicillin in poultry livestock should be restricted. These results will be of great use in the future epidemiological surveillance of *Salmonella* spp. presence and antibiotic resistance among poultry meat and eggs in Turkiye.

Keywords: *Salmonella*, Enteritidis, Infantis, Chicken, Resistance, Tetracycline, Ampicillin

## 1. Introduction

The species *Salmonella enterica* consists of six subspecies with more than 2600 serovars, and among them *S. enterica* subsp. *enterica* is the leading cause of infections in humans and animals (Issenhuth-Jeanjean et al. 2014). *Salmonella* ranked second among the most reported food-associated infections in humans, behind *Campylobacter* in the European Union (EU), and behind norovirus in the United States of America (USA) (Ferrari et al. 2019). According to the EU One Health 2019 Zoonoses Report, 926 salmonellosis outbreaks were reported in 2019 (9,169 illnesses, 1,915 hospitalizations, and seven deaths), which corresponded to 17.9% of all foodborne outbreaks in the EU in the same year. The report also stated that *S. Enteritidis* caused 72.4% of food-borne salmonellosis cases, and the most common salmonellosis associated foods were eggs and egg products (EFSA & ECDC 2021a). Also, as poultry is a well-known reservoir for *Salmonella*, many cases of contaminated poultry meat have been reported to date (CDC 2021). Although poultry meat can be contaminated at any stage from farm to fork, transmission to humans often occurs in food preparation areas because of inadequate sanitation, insufficient cooking, improper storage conditions and/or cross-contamination (Luber 2009). Consequently, poultry meat and eggs are among the main foods involved in the spread of *Salmonella* to humans.

Drug resistance is becoming a big concern, for scientists as humanity nears the edge of the post-antibiotic era. Each year, antimicrobial resistant bacteria account for over 700,000 deaths worldwide, and this number is expected to increase to 10 million by 2050 (O'Neil

2015). In meat production, antibiotic usage is almost inevitable for therapeutic and prophylactic reasons. It is known that poultry do not show any clinical signs when infected by non-typhoidal *Salmonella*, and therefore antibiotic treatment is not required. In this case, however, the bacteria can be exposed to other antibiotics applied to the animals, meant to treat other diseases, and consequently may develop resistance (Voss-Rech et al. 2017). Additionally, although the use of antibiotics for prophylaxis and growth promoter in poultry has been banned in many countries, including Turkey, it is estimated that 60% of all antibiotics produced are used in livestock as they improve the performance effectively and general economics of the production process (Agyare et al. 2018). In this respect, it is important to monitor the antibiotic resistance patterns of *Salmonella* in foods where the pathogen is frequently found. If antibiotic resistance is not monitored well and effectively combated, the list of antibiotics kept as reserve for use in human medicine can be expanded and the number of antibiotics banned for use in veterinary medicine may increase. The prohibition of the use of many antibiotics in veterinary medicine may be on the agenda in the near future, especially colistin, macrolides, fluoroquinolones, and third and fourth generation cephalosporins, which are defined in the publication, WHO Highest Priority Critical Important Antimicrobials, (WHO 2018; European Parliament 2021).

Chicken is the best-integrated farm animal in Türkiye. Approximately 2.2 million tons of broiler meat are produced every year and consumption amounts to 20.5 kg/person (BESD-BIR 2021a; BESD-BIR 2021b). Moreover, in 2018, the number of laying hens in Türkiye reached up to 124 million (TUIK 2021), ranking 8<sup>th</sup> in the world for hen egg production with 1.2 million tones (YUM-BIR 2021). Considering this high amount of production and consumption, the prevalence of *Salmonella* spp. and their antibiotic resistance profiles among chicken meat and eggs has always been studied in Türkiye in some depth. However, the scattered nature of the data complicates the possibility of an all-encompassing interpretation, as these independent studies represent different periods of time and geographic areas. Therefore, we have sought to carry out a meta-analysis in order to determine the pooled prevalence, serotype diversity and antibiotic resistance profiles of *Salmonella* spp. among poultry meat and eggs in Türkiye between 1996 and 2020.

## 2. Material and Methods

### 2.1. Search strategy and study selection

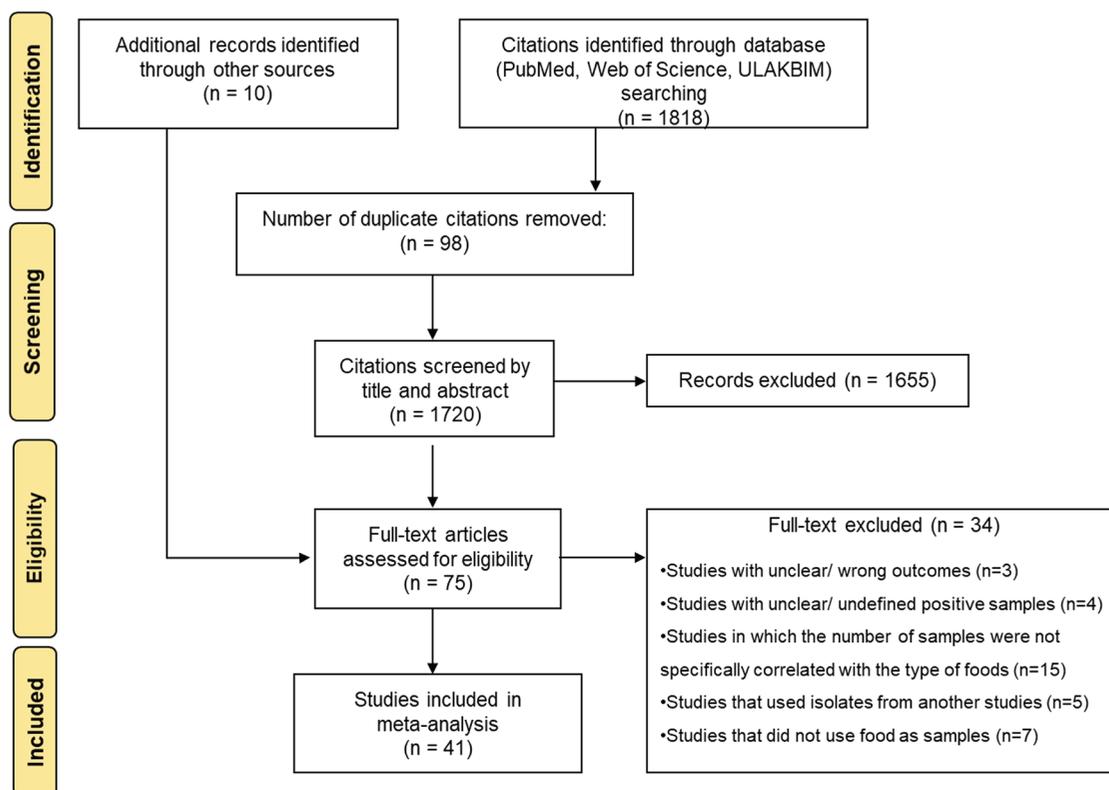
A systematic search was conducted between May and June 2021 using the terms “*Salmonella*” and “Türkiye” or “Türkiye” or “Turkish” and “antimicrobial resistance” or “microbial resistance” or “bacterial resistance” or “resistance pattern” or “resistance” or “susceptibility” or “prevalence” and “chicken” or “poultry” or “broiler” or “turkey” or “egg” in the Web of Science, PubMed and ULAKBIM TR Index databases. Also, a further search was conducted by checking the reference list of relevant papers.

Cross-sectional studies that report the prevalence of any species of *Salmonella* in related foods were included in this systematic review and meta-analysis. Two authors (G.C. and A.N.D.) independently carried out screening in order to identify relevant abstracts and article titles. The advice of the senior author was sought (N.D.A.), as an expert, in case of any disagreement. All the articles were uploaded to Endnote X9.2 (Clarivate Analytics) and duplicates were removed.

Articles written in English or Turkish were included without any date restriction. However, only studies reporting prevalence from Türkiye were included in our study. Reviews, book chapters, letters, theses and conference abstracts were excluded. Additionally, studies with unclear/wrong results, undefined/unclear positive samples, and incomplete information about the prevalence, sample size and/or type of food were also excluded.

### 2.2. Data extraction

One of the authors (A.N.D.) extracted the data for *Salmonella* spp. prevalence among poultry meat and eggs. The second author (G.C.) validated the extracted data according to the systematic literature review flowchart (Figure 1). Any doubts were resolved by consulting the senior author (N.D.A.). The extracted data included author, publication year, city/region, sample source, sampling year, food type, number of samples, number of positive *Salmonella* spp. samples, identified *Salmonella* serovars, antibiotic resistance profiles of *Salmonella* spp. isolates, and the antibiotic susceptibility test method. Accordingly, six food items were tested for their pooled prevalence estimation separately: chicken carcass, chicken parts (drumstick, wing, breast, neck etc.), chicken giblets, chicken ready-to-eat (RTE) foods, turkey meat, and eggs.



**Figure 1- Flowchart of article selection process**

### 2.3. Statistical analyses

The pooled prevalence rates of *Salmonella* spp. among several food types were determined using a fixed and random effects meta-analysis, and the results of these effects are displayed in the manuscript. The effect size measure was determined as the prevalence rate. The distribution of the individual effect and pooled effect sizes were shown with forest plots. On a forest plot, the effects of the individual studies were plotted as boxes with horizontal lines on both sides. The bigger boxes indicate the bigger weights of individual studies and the longer lines indicate the wider confidence intervals (CI). In addition, the diamond at the bottom of the graph presents the combined result. Variations among the trial-level prevalence ratios were evaluated by Q statistics following an  $\chi^2$  distribution with a (k-1) degrees of freedom. The Q statistics were calculated as follows:

$$Q = \sum_{i=1}^k w_i (\hat{\theta}_i - \bar{\theta})^2$$

Where;  $w_i = 1/\sigma_i^2$ ,  $\theta$  is the estimated effect measure and k is the number of studies. In determining the heterogeneity, the  $I^2$  (Inverse variance index) value was used in addition to Q statistics (Higgins & Thompson, 2002). The  $I^2$  value was calculated as follows:

$$I^2 = \frac{Q - (k - 1)}{Q} \times 100\%$$

A value greater than 50% was considered to be high heterogeneity, between 25-50% was considered to be moderate heterogeneity and lower than 25% was considered to be low heterogeneity for the  $I^2$  value (Patsopoulos et al. 2008). The random effects model was accepted when a substantial heterogeneity was obtained according to Q statistics and  $I^2$  value, however the fixed effects model was assumed valid in the opposite case.

The sampling source and sampling year of the studies were taken as sources of heterogeneity. Accordingly, in order to detect the effects of sampling source and sampling year on the overall prevalence rates of *Salmonella* spp. in each food type, a series of meta-regression analyses were conducted. The sampling source variable was defined according to where the samples were obtained, and therefore into three groups: retail markets, farms and slaughterhouses. For meta-regression analysis the retail market was selected as a reference

category. The year variable was described in two groups. The first group was defined as the group for which sampling was done before the last decade, while the second group was defined as the group for which sampling was performed in the last decade. The first group was chosen as the reference category for meta-regression analysis.

Funnel plots were used to assess publication bias. Any asymmetrical scattering of the effect sizes of individual studies and their standard errors were interpreted as evidence of publication bias (Mavridis & Salanti 2014). No funnel plot was created for food groups with less than five studies. Comprehensive Meta-Analysis Version 3.3.070 was used to perform all statistical analysis and graphical representations.  $P < 0.05$  was considered as statistically significant.

### 3. Results

#### 3.1. Description of articles

A total of 1,818 articles from three databases were identified. After removing duplications, and screening the titles/abstracts, 10 studies identified through the reference list of relevant articles were included. Subsequently, the full-texts of 75 papers were examined and 34 of them were excluded because of the reasons that given in Figure 1. Eventually, 41 articles were found eligible for inclusion in this meta-analysis. The studies were published between 1996-2020 and cover a total of 1,451 *Salmonella* spp. positive isolates out of 9,542 isolates. The characteristics of included studies are shown in Supplementary Table 1.

**Table 1- Meta-analysis results of *Salmonella* prevalence**

Food type	<i>Salmonella</i> serovars	% (95% CI) - fixed	% (95% CI) - random	n	I <sup>2</sup>	Q	Sig. of the model
Chicken parts	<i>Salmonella</i> spp.	31.9 (29.8-33.9)	24.4 (17.8-32.6)	24	93.93	378.95*	<0.001 <sup>b</sup>
	<i>Salmonella</i> Infantis	10.9 (8.2-14.4)	6.9 (2.2-20.1)	3	89.77	19.57*	<0.001 <sup>b</sup>
	<i>Salmonella</i> Enteritidis	10.7 (7.7-14.8)	19.0 (3.3-61.6)	2	91.73	12.09*	<0.001 <sup>b</sup>
	<i>Salmonella</i> Typhimurium	19.2 (13.0-27.5)	9.6 (0.9-56.7)	2	93.74	15.98*	<0.001 <sup>b</sup>
	Unknown	22.9 (18.0-28.8)	2.5 (0.5-11.3)	7	93.49	92.22*	<0.001 <sup>b</sup>
Chicken carcass	<i>Salmonella</i> spp.	20.4 (18.7-22.2)	21.9 (14.0-32.7)	13	95.34	257.70*	<0.001 <sup>b</sup>
	<i>Salmonella</i> Infantis	2.5 (1.7-3.7)	2.6 (1.0-6.5)	3	74.48	7.84*	<0.001 <sup>b</sup>
	<i>Salmonella</i> Enteritidis	5.0 (3.5-7.0)	5.8 (2.2-14.4)	4	76.49	12.76*	<0.001 <sup>b</sup>
	<i>Salmonella</i> Typhimurium	5.7 (4.5-7.2)	2.8 (0.8-8.9)	5	93.20	58.86*	<0.001 <sup>b</sup>
	<i>Salmonella</i> Hadar	1.2 (0.7-1.9)	1.2 (0.7-1.9)	3	0.00	1.83	<0.001 <sup>a</sup>
	<i>Salmonella</i> Agona	0.3 (0.1-0.9)	0.3 (0.1-0.9)	2	0.00	0.48	<0.001 <sup>a</sup>
	<i>Salmonella</i> Virchow	0.8 (0.4-1.6)	0.6 (0.1-3.1)	2	77.75	4.45*	<0.001 <sup>b</sup>
Chicken giblets	Unknown	0.5 (0.2-1.5)	0.5 (0.1-2.2)	6	45.66	9.20	<0.001 <sup>a</sup>
	<i>Salmonella</i> spp.	30.0 (25.2-35.2)	20.1 (10.7-34.6)	7	83.77	36.97*	<0.001 <sup>b</sup>
	<i>Salmonella</i> Typhimurium	12.2 (8.2-17.7)	11.3 (5.0-23.7)	2	74.09	3.86*	<0.001 <sup>b</sup>
Chicken RTE food	Unknown	22.9 (18.5-28.0)	22.0 (14.6-31.9)	3	70.87	6.87*	<0.001 <sup>b</sup>
	<i>Salmonella</i> spp.	7.1 (3.3-14.5)	5.4 (1.2-20.9)	3	52.31	4.19*	<0.001 <sup>b</sup>
Egg	<i>Salmonella</i> spp.	11.3 (9.5-13.5)	4.8 (1.7-13.3)	10	95.91	220.10*	<0.001 <sup>b</sup>
	<i>Salmonella</i> Enteritidis	14.0 (11.2-17.4)	22.4 (3.6-69.3)	4	98.11	158.92*	<0.001 <sup>b</sup>
	Unknown	1.0 (0.3-2.8)	0.7 (0.1-4.0)	4	56.56	6.91*	<0.001 <sup>b</sup>
Turkey meat	<i>Salmonella</i> spp.	22.1 (15.0-31.2)	16.7 (5.4-41.1)	3	74.66	7.89*	<0.001 <sup>b</sup>

\*p-value for fixed-effect model, <sup>b</sup>p-value for random-effects model, \*Substantial heterogeneity, CI: Confidence interval, n: Number of the isolates, I: Inverse variance index, Q: Q statistics, sig.: Significance, RTE: Ready-to-eat, spp.: Species

Among the 41 studies, *Salmonella* spp. prevalence was extracted from chicken parts in 24, chicken carcasses in 13, chicken giblets in seven, chicken RTE food in three, eggs in 10 and turkey meat in three studies. Since there is only one study reporting the prevalence of *Salmonella* spp. in quail meat, a meta-analysis could not be performed for this subset.

### 3.2. Pooled prevalence of *Salmonella* spp.

The estimated pooled prevalence rates, heterogeneity findings, and number of studies included in the *Salmonella* spp. meta-analysis across all food types are shown in Supplementary Table 1 and Supplementary Figure 1. Most of the included studies reported the prevalence of *Salmonella* spp. for more than one food group.

In chicken parts, the overall prevalence of *Salmonella* spp. Among the 2,807 isolates was 24.4% (95% CI:17.8-32.6). *Salmonella* Enteritidis had a higher pooled prevalence compared to *S. Infantis* and *S. Typhimurium*. Additionally, the pooled prevalence of unknown serovars among chicken parts was 2.5% (95% CI:0.5-11.3) (Table 1). The effect of year on the pooled prevalence of *Salmonella* spp. was statistically significant according to the random effects meta-regression model ( $p=0.033$ ,  $df=1$ ,  $Q=4.56$ ,  $\tau^2=0.691$ ). The pooled prevalence of the second group [33.7% (95% CI: 21.1-49.1)] was higher than the first group [19.8% (95% CI: 14.4-26.7)]. Nonetheless, the sample source had no effect on the prevalence of *Salmonella* spp. in chicken parts ( $p=0.313$ ,  $df=1$ ,  $Q=1.02$ ,  $\tau^2=0.911$ ).

Among chicken carcasses, the overall prevalence of *Salmonella* spp. among 2,685 isolates was 21.9% (95% CI:14.0-32.7). While the pooled prevalence rates of the identified serovars were observed to be quite low, the highest was found in *Salmonella* Enteritidis with 5.8% (95% CI:2.2-14.4). The pooled prevalence of unknown serovars was found to be 0.5% (95% CI:0.2-1.5) (Table 1). The effect of year and the source of the sample on the pooled prevalence of *Salmonella* spp. was not found to be statistically significant according to the random effects meta-regression model as with the chicken parts ( $p=0.161$ ,  $df=1$ ,  $Q=1.96$ ,  $\tau^2=0.848$  and  $p=0.935$ ,  $df=1$ ,  $Q=0.01$ ,  $\tau^2=1.034$ ).

Among the chicken giblets, the overall prevalence of *Salmonella* spp. Among the 395 isolates was 20.1% (95% CI:10.7-34.6). Among the included studies, *Salmonella* Typhimurium was the only serovar identified in chicken giblets, with a pooled prevalence ratio of 11.3% (95% CI:5.0-23.7). In addition, the unknown serovars' pooled prevalence ratio was found to be 22.0% (95% CI:14.6-31.9) (Table 1). In the meta-regression analysis, year had no significant effect on the pooled prevalence of *Salmonella* spp. ( $p=0.935$ ,  $df=1$ ,  $Q=0.01$ ,  $\tau^2=1.219$ ). The meta-regression analysis could not be performed as all samples of chicken giblets were collected from retail markets in the included studies.

Among eggs, the overall prevalence of *Salmonella* spp. among 3,258 isolates was 4.8% (95% CI:1.7-13.3). Among the included studies, *Salmonella* Enteritidis was the only serovar identified among eggs, with a pooled prevalence rate of 22.4% (95% CI:3.6-69.3). Moreover, the pooled prevalence of unknown serovars was 0.7% (95% CI:0.1-4.0) (Table 1). In the meta-regression analysis, neither the year nor the sample source had a significant effect ( $p=0.857$ ,  $df=1$ ,  $Q=0.03$ ,  $\tau^2=3.233$  and  $p=0.242$ ,  $df=1$ ,  $Q=1.37$ ,  $\tau^2=2.007$ ).

Among chicken, RTE food and turkey meat, the overall prevalence rates of *Salmonella* spp. were 5.4% (95% CI:1.2-20.9) and 16.7% (95% CI:5.4-41.1), respectively. The included studies did not have any serovar identification for these food types (Table 1). Additionally, we were unable to perform meta-regression analysis for these two food types due to the small sample sizes.

Although asymmetrical patterns were observed on the funnel plots, it was not possible to interpret it as concrete evidence of publication bias. Asymmetry in funnel plots might occur by alternative mechanisms such as heterogeneity, small study effect, selective outcome reporting or chance (Supplementary Figure 2).

### 3.3. The pooled prevalence of antimicrobial resistance for *Salmonella* spp.

The results of the meta-analysis of antimicrobial resistance in *Salmonella* spp. are shown in Table 2. The highest resistance was found to clindamycin and oxacillin [0.98 (95% CI:0.92-0.99)], while the lowest resistance was found to be to imipenem and ceftriaxone [0.9% (95% CI:0.1-6.0) and 0.9% (95% CI:0.2-4.3), respectively] among the statistically significant models. The most frequently tested antibiotics were tetracycline, nalidixic acid and ampicillin in the included studies ( $n=13$ ,  $n=12$ , and  $n=12$ , respectively). On the other hand, the less-tested antibiotics were vancomycin, imipenem, meropenem, colistin, ceftiofloxacin, sulfamethoxazole, oxacillin, clindamycin, and enrofloxacin, with two studies available for each antibiotic.

**Table 2- Meta-analysis results of antimicrobial resistance in *Salmonella* species**

<i>Antibiotic</i>	% (95% CI) - fixed	% (95% CI) - random	<i>n</i>	<i>I</i> <sup>2</sup>	<i>Q</i>	<i>Sig. of the model</i>
Tetracycline	61.8 (57.1-66.2)	73.9 (51.0-88.5)	13	95.87	290.31*	0.041 <sup>b</sup>
Nalidixic acid	63.2 (58.2-67.9)	73.4 (48.7-88.9)	12	95.67	253.86*	0.062 <sup>b</sup>
Ampicillin	33.9 (30.5-37.4)	31.5 (20.7-44.6)	12	91.28	126.08*	0.007 <sup>b</sup>
Chloramphenicol	19.9 (16.9-23.2)	14.0 (9.1-21.1)	10	78.11	41.11*	<0.001 <sup>b</sup>
Gentamicin	8.5 (6.6-10.9)	8.8 (4.4-16.9)	10	84.58	58.38*	<0.001 <sup>b</sup>
Streptomycin	67.0 (62.4-71.3)	60.3 (35.5-80.7)	10	95.80	214.29*	0.422 <sup>b</sup>
Trimethoprim-sulfamethoxazole	52.8 (47.6-57.9)	39.6 (18.1-66.0)	9	95.92	195.99*	0.444 <sup>b</sup>
Ciprofloxacin	11.1 (8.4-14.5)	0.11 (5.1-21.9)	8	81.97	38.61*	<0.001 <sup>b</sup>
Kanamycin	35.7 (29.0-43.1)	46.6 (24.9-69.6)	5	87.61	32.28*	0.781 <sup>b</sup>
Trimethoprim	64.3 (59.2-69.0)	73.4 (46.2-89.9)	5	95.64	91.79*	0.088 <sup>b</sup>
Amikasin	13.7 (8.6-21.2)	14.7 (1.8-61.7)	4	93.40	45.47*	0.123 <sup>b</sup>
Cefotaxime	30.0 (25.2-35.5)	16.5 (5.3-40.9)	4	93.82	48.51*	0.011 <sup>b</sup>
Cephalothin	37.2 (30.9-43.9)	33.5 (18.0-53.6)	4	87.22	23.47*	0.106 <sup>b</sup>
Cefazoline	20.4 (16.2-25.5)	15.9 (6.0-36.0)	4	91.93	37.15*	0.003 <sup>b</sup>
Amoxicillin	13.1 (8.8-19.2)	11.5 (4.8-25.0)	4	73.60	11.37*	<0.001 <sup>b</sup>
Neomycin	65.6 (57.8-72.6)	70.4 (55.1-82.1)	4	67.68	9.28*	<0.001 <sup>b</sup>
Erythromycin	87.0 (80.5-91.5)	88.9 (77.9-94.8)	4	56.43	6.87*	<0.001 <sup>b</sup>
Penicillin	68.7 (55.1-79.7)	95.0 (41.1-99.8)	3	87.08	15.48*	0.081 <sup>b</sup>
Sulfonamide	98.2 (93.9-99.5)	98.2 (93.9-99.5)	3	0.00	0.56	<0.001 <sup>a</sup>
Vancomycin	85.9 (77.4-91.5)	87.0 (67.3-95.6)	2	75.44	4.07*	0.002 <sup>b</sup>
Imipenem	0.9 (0.1-6.0)	0.9 (0.1-6.0)	2	0.00	0.29	<0.001 <sup>a</sup>
Meropenem	17.0 (11.8-23.9)	7.8 (0.7-50.3)	2	70.35	3.37*	0.051 <sup>b</sup>
Colistin	2.6 (1.0-6.7)	2.6 (1.0-6.7)	2	0.00	0.44	<0.001 <sup>a</sup>
Cefoxitin	9.2 (6.0-14.1)	6.6 (1.8-21.5)	2	78.42	4.64*	<0.001 <sup>b</sup>
Sulfamethoxazole	45.5 (37.5-53.8)	91.1 (4.4-99.9)	2	93.31	14.95*	0.399 <sup>b</sup>
Oxacillin	97.6 (92.0-99.3)	97.6 (92.0-99.3)	2	0.00	0.37	<0.001 <sup>a</sup>
Clindamycin	97.6 (92.0-99.3)	97.6 (92.0-99.3)	2	0.00	0.37	<0.001 <sup>a</sup>
Ceftriaxone	0.9 (0.2-4.3)	0.9 (0.2-4.3)	2	0.00	0.24	<0.001 <sup>a</sup>
Enrofloxacin	8.3 (0.4-16.4)	8.3 (0.4-16.4)	2	0.00	0.42	<0.001 <sup>a</sup>

\*p-value for fixed-effect model, <sup>b</sup>p-value for random-effects model, \*Substantial heterogeneity, CI: Confidence interval, n: Number of the isolates, I: Inverse variance index, Q: Q statistics, sig.: Significance

#### 4. Discussion

According to the results, the highest pooled prevalence of *Salmonella* spp. belonged to chicken parts with 24.4%. Also, chicken parts were the most studied food type (n=24) and consisted of drumsticks, wings, breasts, necks, skins, and other meat pieces (Supplementary Table 1). In order to interpret this prevalence more accurately, it should be considered together with the pooled *Salmonella* prevalence of chicken carcass, which was found to be 21.9%. These close percentages showed that chicken meat maintains its importance as a risky food for *Salmonella* contamination in Turkiye as well as throughout the world. There are some meta-analyses that report the prevalence of *Salmonella* spp. among chicken meat from another countries and regions. These are as follows: 20% for retail broiler in the USA (Golden & Mishra 2020), 3.2% for chicken meat in Europe (Gonçalves-Tenório et al. 2018), 13.2% for poultry meat/organ in Africa (Thomas et al. 2020), 14% for chicken meat in Ethiopia (Zelalem et al. 2019) and 13.5% for retail chicken in Ethiopia (Tadesse & Gebremedhin 2015). As can be seen, the prevalence in Turkiye is higher than in these countries/regions. Exceptionally, in the study of Golçalvez-Tenório et al. (2018), the overall prevalence among chicken meat was stated to be as high as 58.3% for Turkiye. However, this big difference from our finding is probably due to the very small number of studies that researchers included in their meta-analysis from Turkiye.

Further contamination can occur during slaughtering. In a survey published by EFSA, it was reported that 5% more bacteria were present in the intestines of chickens before slaughter than were found in the carcass at the end of the slaughter line (EFSA 2010). This shows that contamination from carcass to parts is highly probable, especially in integrated plants, where slaughtering and cutting processes take place together. In this context, the very close rates of chicken parts (24.4%) and chicken carcasses (21.9%) in our results can be considered as an indication of the fact that slaughtering and cutting is done in the same facility. Although chicken meat is cooked before consumption, the risk of direct contact with humans and cross-contamination with surfaces or kitchen utensils is high during the pre-cooking process.

Chicken giblets have been associated with *Salmonella* outbreaks several times in many countries (CDC 1984; CDC 2012; Lanier et al. 2018). The chicken liver is considered to be one of the more common locations for foodborne infections. The Food Safety and Inspection Service (2022) highly recommends consuming chicken liver dishes after being cooked to an internal temperature of 74 °C as pathogens can exist both on the external surface and in the internal parts of the liver (FSIS 2021). In this meta-analysis study, the *Salmonella* prevalence rate was found to be 20.1%, which is almost as high as in chicken parts and carcasses. This rate can be explained by cross-contamination during slaughtering, thus much more care needs to be taken while handling chicken.

The pooled *Salmonella* prevalence of chicken RTE food and turkey meat were found 5.4% and 16.7%, respectively. However, the low number of the studies (two for chicken RTE food and three for turkey meat) prevents the interpretation of these ratios accurately. Nevertheless, when compared with the 2019 EFSA report, these rates are quite higher than the mean incidence of *Salmonella* spp. in RTE foods (0.27%) and fresh turkey meat (5.3%) (EFSA & ECDC 2021a).

Eggs have a distinct place among other foods in terms of the way it is contaminated with *Salmonella*. Two possible routes for the *Salmonella* contamination of eggs were defined; horizontal (penetration through the eggshell) and vertical (transovarian, direct contamination of the inner egg while passing through the reproductive organs) (Cardoso et al. 2021). Therefore, some studies both investigate egg contents and eggshells. However, because of the low number of egg studies conducted in Türkiye, the presence of *Salmonella* in eggs was included as a whole in this meta-analysis. Thus, the pooled *Salmonella* prevalence for eggs was determined to be 4.8%. This ratio is quite higher than in the EFSA report (0.13%) (EFSA & ECDC, 2021a). On the other hand, Hosseinezhad et al. (2020) report an overall prevalence of *Salmonella* in eggs of 6.89% in Iran, which is almost the same as our result. Diker et al. (2020) report the prevalence of *Salmonella* spp. among table eggs to be 3.3% (24/726), when purchased from varied regions in Türkiye. Since this prevalence was obtained from a project carried out within the scope of Türkiye's national *Salmonella* control program, it is noteworthy that both of the reported figures are close to each other. However, although the prevalence for eggs is not as high as in chicken parts and carcasses, it is essential to reduce this rate, considering that Türkiye ranks 3<sup>rd</sup> in the world for egg exports (YUM-BIR 2021).

*Salmonella* Enteritidis was found to be the most common serotype among eggs, chicken parts and chicken carcasses with the rates of 22.4%, 19.0% and 5.8%, respectively. The results are not surprising because *S. Enteritidis* is considered to be the main serotype associated with both human salmonellosis (Ferrari et al. 2019) and infections associated with foodborne outbreaks including eggs (Cardoso et al. 2021). Moreover, in the Turkish Food Codex Regulation on Microbiological Criteria "*Salmonella* spp.," which is required to be checked for in raw poultry meat and prepared poultry meat mixtures, was changed to "*S. Enteritidis* and *S. Typhimurium*" in 2018 (Turkish Food Codex 2018). On the other hand, according to the results of the project carried out to develop a national *Salmonella* monitoring program (TUBITAK 2017), *S. Kentucky* was found as the predominant serotype detected in all of the sample matrices collected from laying hens, while *S. Infantis* was reported to be the dominant serotype in broilers, slaughterhouses, breeder flocks, turkey meat and food samples in Türkiye. It is thought that the most probable reason for this incompatibility between the results of the nationwide project and this meta-analysis is due to the scarce and scattered studies on prevalence conducted based on *Salmonella* in chicken meat and eggs. Also, the difference between the predominant serotypes is probably due to the inclusion of studies conducted in the last 24 years. Undoubtedly, the serotype distribution has started to change in recent years and *S. Infantis* is becoming the most frequently isolated serotype in poultry in Türkiye. Moreover, as it can be seen in Table 1 only a few studies report *Salmonella* serotypes in foods, and the prevalence of unknown serotypes is noteworthy. It can be observed that most of the included studies presented serotyping for *S. Enteritidis* and *S. Typhimurium* specifically, but only two of the studies further investigated *Salmonella* spp. isolates using a Kauffmann-White classification (Supplementary Table 1). Therefore, more comprehensive studies should be performed in order to determine the *Salmonella* serotype distribution in Türkiye.

The highest pooled antibiotic resistance prevalence of *Salmonella* spp., regardless of food type belonged to tetracycline and ampicillin with 73.9% and 31.5%, respectively (Table 2). Although the percentages were found to be quite higher for sulfonamide, erythromycin and vancomycin, the prevalence of these antibiotics was not taken into account due to the small number of studies on the topic. Also,

resistance to streptomycin (60.3%) and trimethoprim-sulfamethoxazole (39.6%) were found to be high but not significant ( $p>0.05$ ). Tetracycline, ampicillin and sulfamethoxazole are widely used in veterinary medicine for the treatment of infections in food-producing animals. High resistance to tetracycline, sulfamethoxazole and ampicillin of *Salmonella* spp. were also found at an alarming level in the 2018/2019 EFSA report (EFSA & ECDC 2021b). According to data obtained from 28 EU Member States ampicillin, sulfamethoxazole and tetracycline resistance was reported to be 13.7%, 33.9% and 35.5% for broiler carcasses, respectively. Notably, a very high level of tetracycline (57.3%) in Turkish carcasses and a high level of resistance to nalidixic acid (48.8%) was reported. In the meta-analysis of Voss-Rech et al. (2017), the highest resistance of poultry-related *Salmonella* isolates was found for nalidixic acid, sulfonamide, and tetracycline with 48.2%, 43.8% and 32%, respectively.

In this study, the pooled prevalence of *Salmonella* spp. was compared for the last two decades by year. A significant increase was observed in pooled chicken parts prevalence after 2011, from 19.8% to 33.7%. Although the prevalence is expected to decrease due to stricter rules and intensified controls, with changes made to regulations in the last decade, it is thought that the reason for this dramatic increase is mainly due to the more sensitive and advanced laboratory methods used in the detection of foodborne pathogens (Cufaoglu, Ambarcioglu et al. 2021).

## 5. Conclusions

To the best of our knowledge, this is the first meta-analysis study on the prevalence, serotype diversity and antibiotic resistance profiles of *Salmonella* spp. isolated from poultry meat and eggs in Türkiye. The high-pooled prevalence of the organism highlighted the potential threat *Salmonella* poses for public health. Additionally, findings related to the antibiotic resistance profiles of *Salmonella* spp. show that the tetracycline, quinolones and penicillin groups of antibiotics used with poultry livestock should be restricted. Overall, the general lack of research makes it difficult to estimate the genuine prevalence of *Salmonella* among poultry meat and eggs. Moreover, the lack of evidence on *Salmonella* serotypes has impeded our attempt at a comprehensive outcome. Therefore, a more comprehensive studies on the presence and serotype distribution of the pathogen in foods is required within the framework of the Turkish national *Salmonella* control program.

**Data availability:** Data are available on request due to privacy or other restrictions.

**Authorship Contributions:** Concept: G.C., N.D.A., Design: G.C., Data Collection or Processing: G.C., P.A., A.N.D., Analysis or Interpretation: G.C., P.A., N.D.A., Literature Search: G.C., A.N.D., Writing: G.C., P.A., N.D.A.

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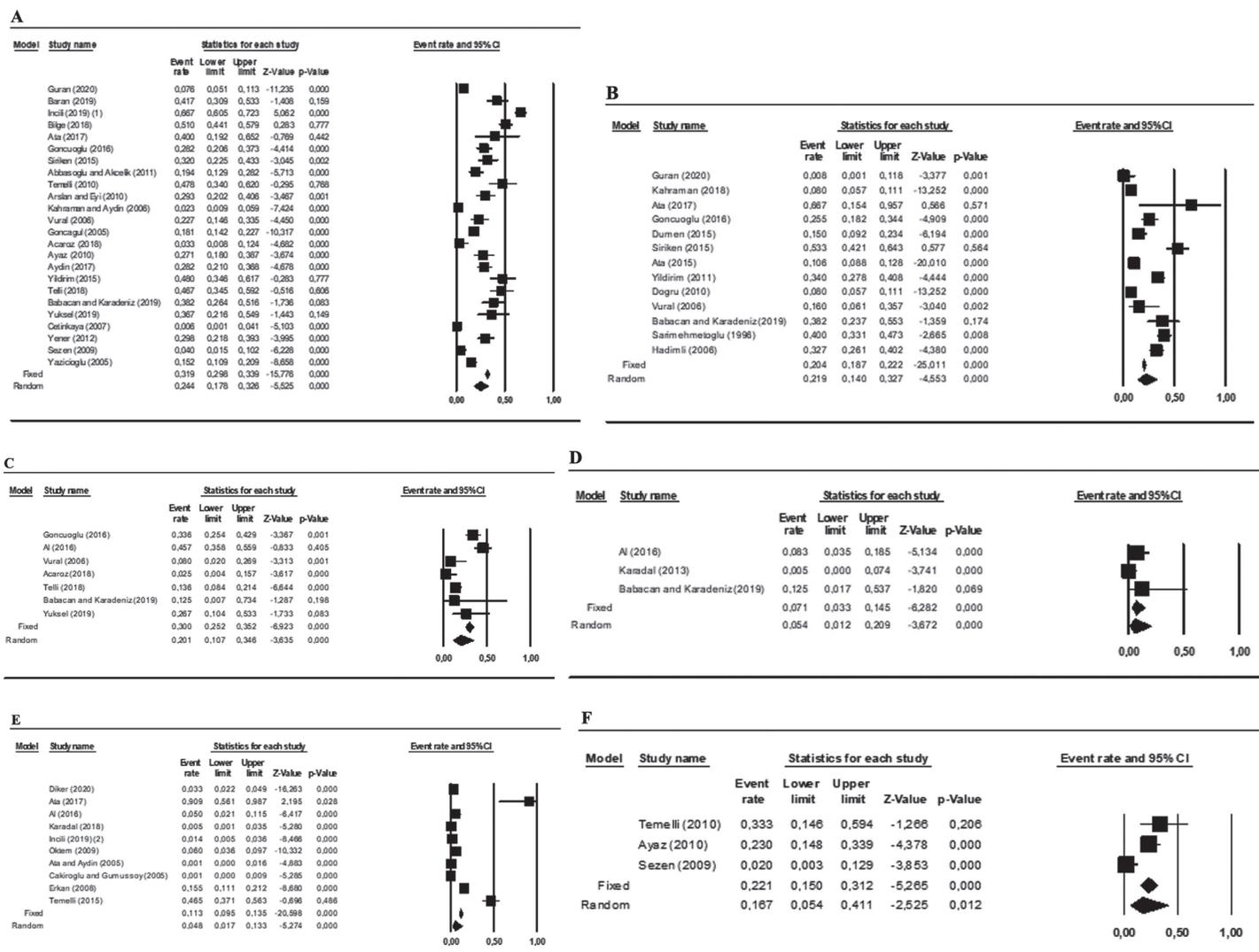
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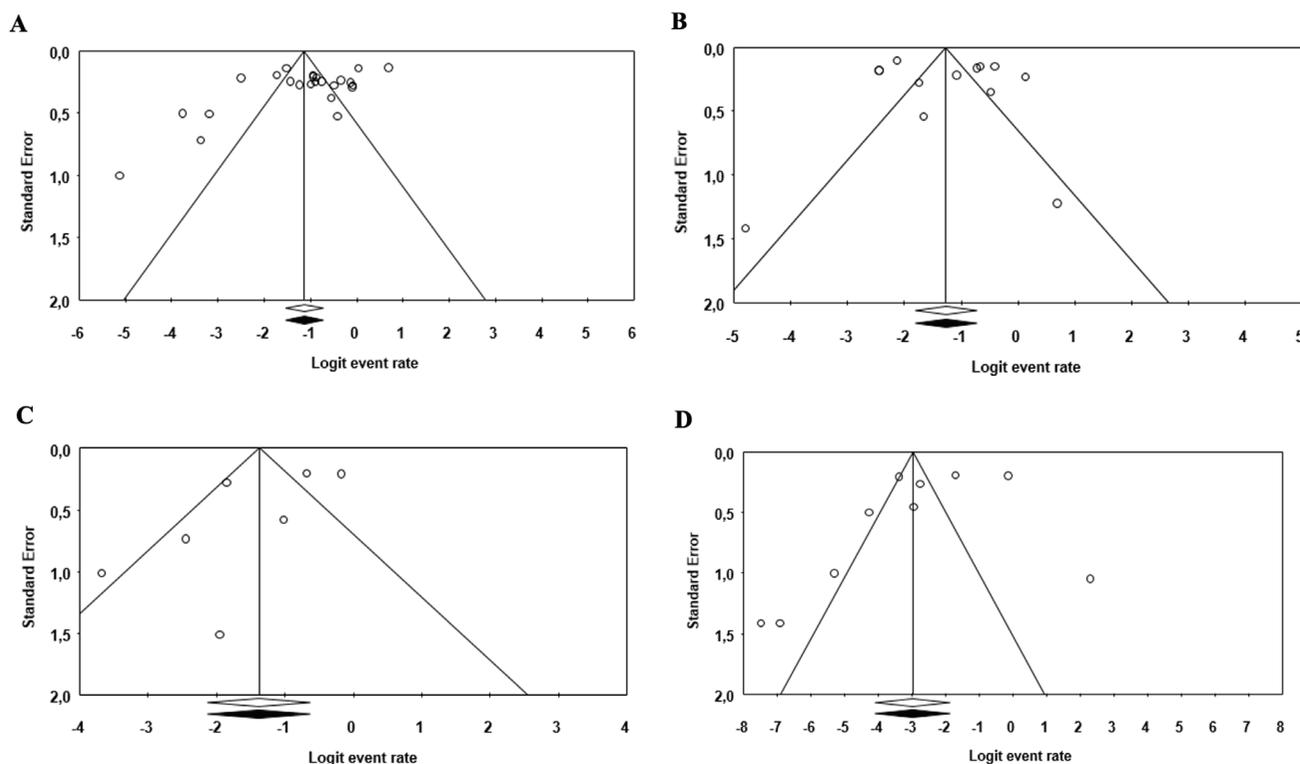
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**Supplementary Figure 1- Forest plots of the prevalence of *Salmonella* spp. in food types. Black boxes: effect size of each study; horizontal lines: 95% confidence interval; diamonds: overall effects for fixed and random effect models. (a) chicken parts, (b) chicken carcass, (c) chicken giblets, (d) chicken RTE food, (e) egg, (f) Turkey meat**



**Supplementary Figure 2- Funnel plots of the prevalence of *Salmonella* spp. in food types. Dots: individual studies; triangular regions: 95% confidence interval area; vertical lines: overall effect. (a) chicken parts, (b) Chicken carcass, (c) chicken giblets, (d) egg, chicken RTE food contains less than five studies, turkey meat contains less than five studies**

**Supplementary Table 1- Characteristics of included studies**

<i>Author/year</i>	<i>Sample source</i>	<i>Sampling year</i>	<i>Food type</i>	<i>Number of samples</i>	<i>Salmonella spp. positive samples</i>	<i>Identified serovars</i>	<i>Antibiotic resistance profiles of Salmonella isolates</i>	<i>Antibiotic susceptibility test method</i>
Abbasoglu & Akcelik 2011	Retail markets	-	Chicken meat	103	20	<i>S. Infantis</i>	KAN, TET, NEO, SPEC, S, NA, TMP,	Disk diffusion
Acaröz et al. 2018	Retail markets	June-December 2017	Chicken parts Chicken giblets	100	3	-	-	-
Al et al. 2016	Retail Markets and Farms	July - August 2014	Egg Chicken RTE Chicken giblets	252	52	<i>S. Typhimurium</i> <i>S. Enteritidis</i>	AMP, TET, AMC, CFZ, ERY, GEN, NEO, NA, ENR, STX	Disk diffusion
Arslan & Eyi 2010	Retail markets	-	Poultry meat	75	22	<i>S. Typhimurium</i> <i>S. Bongori</i>	-	-
Ata & Aydin 2008	Farms	-	Egg	500	-	-	-	-
Ata et al. 2015	Slaughterhouse	January 2008 - January 2010	Chicken carcass	930	99	<i>S. Typhimurium</i> , <i>S. Infantis</i> , <i>S. Hadar</i> , <i>S. Branderburg</i> , <i>S. Kentucky</i> , <i>S. Corvallis</i> , <i>S. Agona</i> , <i>S. Chincol</i> , <i>S. Dabou</i> , <i>S. Emek</i> , <i>S. Essen</i> , <i>S. Seftenberg</i> , <i>S. Kingston</i> , <i>S. Virchow</i>	AMP, TET, AMC, NA, C AZT, STX, GEN, CEF	Disk diffusion

Supplementary Table 1- Continued

<i>Author/year</i>	<i>Sample source</i>	<i>Sampling year</i>	<i>Food type</i>	<i>Number of samples</i>	<i>Salmonella spp. positive samples</i>	<i>Identified serovars</i>	<i>Antibiotic resistance profiles of Salmonella isolates</i>	<i>Antibiotic susceptibility test method</i>
Ata et al. 2017	-	2000 - 2015	Chicken parts Egg	29	18	<i>S. Enteritidis</i>	-	-
Ayaz et al. 2010	Retail markets	June 2008 - May 2009	Chicken parts	214	36	<i>Salmonella</i> spp.	-	-
Aydın, 2017	Retail markets	-	Chicken parts	124	35	<i>Salmonella</i> spp.	-	-
Babacan and Karadeniz, 2019	Retail markets	-	Chicken parts Chicken RTE	100	35	<i>Salmonella</i> spp.	S, AMS, STX, NEO, OXT, TET, OFL, FLO, AMX, C, CIP, DOX, ENR	Disk diffusion
Baran et al. 2019	Retail markets	May- December 2016	Chicken parts	72	30	<i>Salmonella</i> spp.	STR, NA, CIP, TMP, C, GEN, STX, AMP, KAN, TET	Disk diffusion
Bilge et al. 2018	Retail markets	March- August 2017	Chicken parts	200	102	<i>Salmonella</i> spp.	GEN, CEF, AMP, C, CFZ, CTX, CIP, STR, STX, TET, NA, TMP	Disk diffusion
Cakıroğlu & Gümüşsoy, 2005	-	July- December 2003	Egg	882	0	-	-	-
Cetinkaya et al. 2008	Retail markets	December 2004 - June 2005	Chicken parts	168	1	<i>S. Infantis</i>	NA, STR, S, TET, TMP, STX	Disk diffusion
Diker et al. 2020	Farms	2015 - 2017	Egg	726	24	<i>S. Enteritidis</i> , <i>S. Salamae</i>	-	-
Dümen et al. 2015	Retail markets	-	Chicken carcasses	100	15	<i>S. Enteritidis</i> , <i>S. Typhimurium</i>	-	-
Erkan et al. 2008	Retail markets	-	Egg	200	31	-	-	-
Goncagül et al. 2005	Retail markets	-	Chicken parts	315	57	<i>S. Enteritidis</i>	-	-
Goncuoglu et al. 2016	Retail markets	January 2009 - March 2012	Chicken parts Chicken carcass Chicken giblets	330	96	<i>S. Typhimurium</i>	AMC, FOX, IPM, AMP, C, CFZ, STX, TET, NA, GEN, KAN, EFT, CIP, SUL, TMP, CFZ, AK, CRO, STR, S	Disk diffusion
Guran et al. 2020	Retail markets	December 2016 - April 2018	Chicken parts Chicken carcass	348	22	<i>S. Infantis</i>	AK, AMC, AMP, CIP, CT, GEN, NET, TGC, STX	Phoenix NMIC- 400/ID Panel
Hadimli, 2006	Retail markets	-	Chicken carcass	168	55	<i>Salmonella</i> spp.	-	-
İncili et al. 2019	Slaughterhouse	October 2013 - July 2014	Chicken parts	240	160	<i>Salmonella</i> spp.	AMP, CIP, NA, STR, GEN, SUL, TMP, C, TET, CT, CTX, CEF, FOX, CFP, MEM	Disk diffusion
İncili et al. 2019	Retail markets	January- December 2018	Egg	288	4	<i>Salmonella</i> spp.	-	-

Supplementary Table 1- Continued

<i>Author/year</i>	<i>Sample source</i>	<i>Sampling year</i>	<i>Food type</i>	<i>Number of samples</i>	<i>Salmonella spp. positive samples</i>	<i>Identified serovars</i>	<i>Antibiotic resistance profiles of Salmonella isolates</i>	<i>Antibiotic susceptibility test method</i>
Kahraman et al. 2018	Retail markets	July 2014 - December 2016	Chicken carcass	400	32	<i>Salmonella</i> spp.	AMP, CTX, MEM, IMP, KAN, STR, NA, CIP, TET, C, ERY, STX	Disk diffusion
Kahraman and Aydin, 2009	Retail markets	March 2007 - February 2008	Chicken parts	175	4	<i>Salmonella</i> spp.	-	-
Karadal et al. 2013	Retail markets	September-December 2012	Chicken RTE	100	0	-	-	-
Karadal et al. 2018	Retail markets	-	Egg	200	1	<i>Salmonella</i> spp.	-	-
Kasimoglu-Dogru et al. 2010	Retail markets	2003 - 2005	Chicken carcass	400	32	<i>S. Enteritidis</i> , <i>S. Vircho</i> , <i>S. Typhimurium</i> , <i>S. Hadar</i>	PG, NA, CEP, STR, TET	Disk diffusion
Öktem et al. 2009	Retail markets	-	Egg	250	15	<i>S. Enteritidis</i>	-	-
Sarimehmetoğlu et al. 1996	Slaughterhouse	May - July 1995	Chicken carcass	180	72	-	-	-
Sezen, 2009	Retail markets	February -June 2007	Chicken parts Turkey meat Quail meat	175	6	<i>Salmonella</i> spp.	-	-
Siriken et al. 2015	Retail markets	2008 - 2009	Chicken carcass Chicken parts	150	64	<i>Salmonella</i> spp.	GEN, VAN, C, STR, CRO, TET, NA, AMP, STX	Disk diffusion
Telli et al. 2018	Retail markets	January 2015 - January 2017	Chicken parts Chicken giblets	170	43	<i>S. Enteritidis</i> , <i>S. Typhimurium</i>	AK, AMP, CEP, CEZ, CIP, CLI, C, ERY, GEN, KAN, NA, OX, P, STX, TEL, TET, VAN	Disk diffusion
Temelli et al. 2010	Retail market	-	Chicken parts	61	27	-	-	-
Temelli et al. 2015	Retail markets	-	Egg	101	47	<i>S. Enteritidis</i>	-	-
Vural et al. 2006	-	-	Chicken carcasses Chicken parts Chicken giblets	125	23	<i>Salmonella</i> spp.	-	-
Yazıcıoğlu et al. 2005	Slaughterhouse	-	Chicken parts	197	30	<i>S. Virchow</i> , <i>S. Bsilla</i> , <i>S. Enteritidis</i> , <i>S. Typimurium</i>	NA, STR	Disk diffusion
Yener et al. 2012	Retail markets	-	Chicken parts	104	31	-	-	-
Yildirim et al. 2011	Retail markets	April 2005 - March 2006	Chicken carcass	200	68	<i>S. Typhimurium</i> , <i>S. Infantis</i> , <i>S. Heidelber</i> , <i>S. Hadar</i> , <i>S. Enteritidis</i> , <i>S. Newport</i> , <i>S. Thompson</i> , <i>S. Montevideo</i> , <i>S. Agona</i> , <i>S. Ohio</i> , <i>S. Rough</i> , <i>S. Strain</i>	PG, OX, CLI, VAN, ERY, AMP, TET, STR, NEO, CEP, GEN, C, CTX, AK	Disk diffusion

Yildirim et al. 2015 Retail markets - Chicken parts 50 24 *Salmonella* spp. - -

Supplementary Table 1- Continued

Author/year	Sample source	Sampling year	Food type	Number of samples	Salmonella spp. positive samples	Identified serovars	Antibiotic resistance profiles of Salmonella isolates	Antibiotic susceptibility test method
Yüksel et al. 2019	Retail markets	-	Chicken parts Chicken giblets	90	15	<i>Salmonella</i> spp.	CIP, STX, C, STR, AMP, GEN, KAN, NA, TMP, TET	Disk diffusion

AK: Amikacin, AMC: Amoxicillin/clavulanic acid, AMP: Ampicillin, AMS: Ampicillin sulbactam, AZT: Aztreonam, CFZ: Cefazoline, CFP: Cefepime, CE: Ceftazidime, EFT: Ceftiofur, FOX: Cefoxitin, CTX: Cefotaxime, CEF: Cefoperazone, CRO: Ceftriaxone, CEP: Cephalothin, CEZ: Cephazolin, C: Chloramphenicol, CIP: Ciprofloxacin, CLI: Clindamycin, CT: Colistin, DOX: Doxycycline, ENR: Enrofloxacin, ERY: Erythromycin, FLO: Florphenicol, GEN: Gentamicin, IPM: Imipenem, KAN: Kanamycin, MEM: Meropenem, NA: Nalidixic acid, NEO: Neomycin, NET: Netilmicin, OFL: Ofloxacin, OX: Oxacillin, OXT: Oxytetracycline, PG: Penicillin G, STR: Streptomycin, SPEC: Spectinomycin, SUL: Sulfamethoxazole, S: Sulphonamide, TEI: Teicoplanin, TET: Tetracycline, TGC: Tigecycline, TMP: Trimethoprim, STX: Trimethoprim-sulfamethoxazole, VAN: Vancomycin

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