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Investigation of the Presence of Extended Spectrum Beta Lactamase, Carbapenem and Colistin Resistances in *Salmonella* spp. Isolates by Phenotypic Method and PCR

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ABSTRACT

The study was aimed to investigate extended-spectrum beta lactamase (ESBL) and carbapenem resistance by phenotypically and genotypically in *Salmonella* spp. strains isolated from stool samples of free-range chickens between August 2018 and January 2020. Also it was aimed to investigate colistin resistance gene mcr-1 by PCR. Stool samples collected from flocks' ground from 110 different family-type chicken flocks by veterinarians about at least 25 grams of each flocks into the sterile containers for microbiological examinations in regions and villages of Balıkesir province. *Salmonella* spp. isolation were done according to ISO 6579-2017 method. A total of 34 *Salmonella* spp. isolates were identified based on biochemical characterization and confirmed by PCR targeting the *16S rRNA* gene. ESBL and carbapenem resistance investigated by conventionally according to EUCAST methods. The presence of *blaTEM, blaSHV, blaCTX-M, IMP, OXA-48 like, NDM, KPC* resistance genes was investigated using the polymerase chain reaction (PCR) method. Colistin resistance was investigated by PCR method to determine the presence of mcr-1 gene. One isolate (2.94%) showing a meropenem inhibition zone <25 mm and resistance to piperacillin/tazobactam was classified as carbapenem-resistant. As a result of serotyping of carbapenem resistant isolate was identified as *Salmonella* Infantis. PCR analysis revealed the presence of both the *IMP* and *OXA-48-like* resistance genes in this strain. Phenotypic characterization supported the genotypic findings were resistance to temocillin indicated *OXA-48-like* carbapenemase activity, while the detection of a synergistic effect with dipicolinic acid (DPA) in the combined disk diffusion assay confirmed the functional expression of a metallo-β-lactamase (MBL) associated with the *IMP* gene. To the best of our knowledge, this study represents the first report of the detection of carbapenem resistance in *Salmonella* spp. isolates, isolated from chicken stool in Türkiye. The presence of carbapenem resistance was detected in one *Salmonella* isolate, serotyped as *S.* Infantis.

INTRODUCTION

Carbapenemases are β-lactamase enzymes capable of hydrolyzing a broad spectrum of β-lactam antibiotics, including penicillins, most cephalosporins, carbapenems, and, in some cases, monobactams. Unlike metallo-β-lactamases (MBLs), certain carbapenemases are not inhibited by metal ion chelators. These enzymes represent a significant clinical threat due to their ability to confer resistance to nearly all β-lactam antibiotics and their potential for horizontal gene transfer. Moreover, carbapenemase-producing bacteria often harbor additional resistance mechanisms, resulting in multidrug-resistant phenotypes. Infections caused by such organisms, particularly those belonging to the *Enterobacteriaceae* family, are associated with limited treatment options and high mortality rates (EUCAST, 2017B).

Carbapenem-resistant Enterobacteriaceae (CRE) have been designated as a Priority 1 – Critical pathogen group

in the World Health Organization's 2017 list of antibiotic-resistant bacteria for which the development of new antimicrobial agents is urgently required (WHO, 2017).

Among the various carbapenemases, OXA-48 is currently the most rapidly disseminating type across Europe, with reports of regional outbreaks in several countries. IMP-type carbapenemases, while less prevalent in Europe, are widely distributed in other parts of the world. The clinical significance of carbapenemases lies in their ability to confer resistance to nearly all β-lactam antibiotics, their high potential for horizontal gene transfer, and their frequent co-occurrence with other resistance determinants, leading to multidrug-resistant phenotypes. Infections caused by carbapenemase-producing organisms are associated with limited treatment options and are often linked to high morbidity and mortality rates (EUCAST, 2017b).

Colistin, long regarded as a last-resort antibiotic for the treatment of infections caused by multidrug-resistant Gram-negative bacteria, has increasingly lost its clinical effectiveness due to the emergence of resistance. This resistance arises through chromosomal mutations as well as the acquisition of plasmid-mediated resistance determinants, most notably the mobilized colistin resistance (mcr) genes. The first mcr gene, mcr-1, was initially identified in *Escherichia coli* isolated in China in 2016. Since then, numerous studies have reported a growing diversity of mcr variants, currently ranging from mcr-1 to mcr-10, predominantly among members of the *Enterobacteriaceae* family across different regions of the world. The rapid global dissemination of these plasmid-borne colistin resistance genes represents a serious and escalating threat to public health, significantly compromising the therapeutic value of one of the last available treatment options against critical Gram-negative infections (Mondal et al., 2024).

Salmonella infections are among the zoonotic diseases that cause significant economic losses in poultry farming by leading to reduced productivity and high mortality rates (Babacan and Karadeniz, 2019; Hossain et al., 2021; Yıldırım et al., 2022). These bacteria can also cause infections in humans. *Salmonella* bacteria belong to the family *Enterobacteriaceae* (Babacan and Karadeniz, 2019; Hossain et al., 2021). Chickens are the primary source of transmission for Salmonella infections in humans, and infected animals can transmit the bacteria to humans through the food chain. When these infections are spread through food, they pose a significant public health threat, and Salmonella outbreaks can occur from the consumption of contaminated food. In recent years, there has been a notable increase in the prevalence of zoonotic gastrointestinal diseases worldwide, with *Salmonella* species being more frequently isolated compared to other animals and animal-derived foods. The rise in poultry consumption has also contributed to the increased prevalence of poultry-related zoonotic diseases. *Salmonella* serovars can cause acute or chronic, often subclinical infections in poultry, leading to food poisoning in humans (Akgül et al., 2021; Kırkan et al., 2017; Lozano-Villegas et al., 2024; Quinn et al., 2004; Salar et al., 2015). Monitoring antibiotic resistance in zoonotic and commensal bacteria is highly important for understanding the development and spread of resistance. Therefore, control programs targeting poultry flocks are being implemented in Europe, and in our country, the National Salmonella Control Program was initiated by the Ministry of Agriculture and Forestry in 2018 (Republic of Türkiye, Ministry of Agriculture and Ministry, 2018).

Antibiotics are commonly used in the treatment of Salmonella infections, which contributes to the development of antibiotic resistance. Notably, serovars such as *S.* Enteritidis, *S.* Typhimurium, *S.* Kentucky, and *S.* Infantis can cause infections in humans and are closely monitored for antibiotic resistance. The spread of especially multidrug-resistant *S.* Infantis and *S.* Kentucky strains should be carefully tracked, as *S.* Kentucky shows high resistance to ciprofloxacin. (Akgül et al., 2021; Bénao et al., 2024; Sevük Akkaya and Ak, 2023; Şahan et al., 2016; Waktole et al., 2024).

Animals, particularly poultry, serve as important reservoirs in the transmission of antimicrobial-resistant bacteria to humans, raising critical concerns for food safety and public health. Numerous studies have documented the emergence of carbapenem-resistant *Salmonella* strains isolated from both human clinical specimens and poultry-related sources. (Abdel-Kader et al., 2022).

In both developed and developing countries, the multidrug resistance of *Salmonella* strains isolated from poultry meat poses a serious risk to human health. The transmission of antibiotic-resistant bacteria to humans through food threatens public health and can lead to the transfer of resistance genes to other pathogens. Therefore, the proper use of antibiotics in poultry and the implementation of appropriate preventive health measures are of great importance. The use of drugs that do not lead to antibiotic residues can help prevent the development of multidrug resistance. However, the ease with which antibiotic resistance genes can be transferred between bacteria further complicates the situation (Kırkan et al., 2017; Kutu, 2017; Lozano-Villegas et al., 2024; Şahan et al., 2016; Temelli et al., 2012).

The study was aimed to assess extended- spectrum beta lactamase and carbapenem resistance by phenotypically and genotypically in *Salmonella* strains isolated from stool samples of free-range chickens between August 2018 and January 2020. Also it was aimed to investigate colistin resistance gene *mcr-1* by PCR, according to the multiplex pcr method for carbapenem resistance genes include *mcr-1*.

MATERIALS AND METHODS

Materials and Sampling

Stool samples collected from flocks' ground from 110 different family-type chicken flocks by veterinarians about at least 25 grams of each flocks into the sterile containers for microbiological examinations in different regions and villages of Balıkesir province between August 2018 and January 2020. Samples were delivered to the laboratory under cold chain conditions by veterinarians.

Isolation and Identification of Salmonella spp.

Salmonella spp. isolation were done according to ISO 6579-2017 (ISO, 2017) method from chicken stools. Then, the suspicious colonies growing on XLT_4 agar (Merck, Germany) were identified as *Salmonella* spp. with biochemical tests (Gram negative rod, oxidase negative, catalase positive, H_2S positive, indol negative, gas positive, glikoz fermentative, lactose and sucrose non-fermentative, urease negative, metil red test positive, Voges Preskauer test negative) (ISO, 2017; Quinn et al., 2004) and genus-specific primers targeting the *16S rRNA* gene of *Salmonella enterica* were previously described by Mir et al. (2015) (Table 1), along with amplification conditions (Mir et al., 2015), by PCR method. The serotype of a *Salmonella* spp. isolate, which exhibited carbapenem resistance determined phenotypically and by

PCR (ISO, 2017; Mir et al., 2015; Quinn et al., 2004), was identified through serotyping according to ISO 6579-2017 (ISO, 2017).

To obtain pure cultures for DNA extraction, all *Salmonella* spp. isolates previously identified through biochemical testing were streaked out into Nutrient Broth (NB, Oxoid, UK) and incubated at 37°C for 18 hours. Following incubation, 1 mL of each culture was centrifuged at 5000 × g for 10 minutes. The supernatant was discarded, and genomic DNA was extracted from the resulting pellet using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA), following the manufacturer's protocol optimized for Gram-negative bacteria. Extracted DNA samples were stored at −20°C until further use in PCR for other molecular analyses.

PCR amplification of the *Salmonella enterica 16S rRNA* gene was performed in a total reaction volume of 25 µL. Each reaction included 5 µL of template DNA and 20 µL of PCR master mix, which consisted of 12.5 µL of DreamTaq PCR Master Mix (2X) (Thermo Scientific, USA), 7.3 µL of DEPC-treated water, 0.1 µL of forward primer (100 pmol/µL), and 0.1 µL of reverse primer (100 pmol/µL). PCR cycling conditions were applied according to the protocol described by Mir et al. (2015), and primer sequences are listed in Table 2.

PCR products (10 µL of amplicon mixed with 2 µL of 10X BlueJuice gel loading buffer; Thermo Scientific, USA) were separated via electrophoresis on 1.5% agarose gels (Prona) prepared in 1X Tris-Borate-EDTA (TBE) buffer. DNA fragments were visualized using a gel documentation system (EBOX CX5 TS EDGE, Vilber). A 100 bp DNA ladder (GeneRuler 100 bp, Thermo Scientific, USA) served as a molecular size marker.

Phenotypic Detection of Extended-Spectrum Beta Lactamase and Carbapenem Resistance

To determine the presence of extended-spectrum beta-lactamase and carbapenem resistance in isolates identified as *Salmonella* spp. through biochemical tests and PCR, conventional methods outlined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Jean et al. (2015) were done (EUCAST, 2017a; EUCAST, 2017b; Jean et al., 2015). According to the EUCAST and Jean et al. (2015) methods, a combined disk diffusion test was performed using cefotaxime 5µg (Liofilchem, Italy), ceftazidime 10µg (Liofilchem, Italy), cefpdoxime 10µg (Liofilchem, Italy), cefotaxime-clavulanic acid 40µg (Liofilchem, Italy) and cefepime 30µg (Liofilchem, Italy), cefepime-clavulanic acid 40µg (Liofilchem, Italy) disks to determine the presence of ESBL. The results of disc diffusion test and inhibition zone diameters were evaluated diameters were evaluated according to the EUCAST standards and Jean et al. (2015) (EUCAST, 2017a; EUCAST, 2017b; Jean et al., 2015).

According to the EUCAST method, a disk diffusion test was performed using meropenem 10µg (Liofilchem, Italy), meropenem + phenylboronic acid (Liofilchem, Italy), meropenem + dipicolinic acid (DPA) (Liofilchem, Italy), meropenem + cloxacillin (Liofilchem, Italy), temocillin 30µg (Liofilchem, Italy), piperacillin-tazobactam 110µg (Oxoid, UK) disks to determine carbapenem resistance. Except for piperacillin-tazobactam, the results were evaluated according to the EUCAST standards and van Dijk et al. (2014). (EUCAST, 2017a; EUCAST, 2017b; van Dijk et al., 2014). Piperacillin- tazobactam results was evaluated according to CLSI guideline (CLSI, 2020).

Genomic DNA Extraction and Detection of Antimicrobial Resistance Genes via PCR

Additionally, PCR was performed using specific primers and amplification conditions described by Bektaş et al. (2018) for ESBL genes to detect the presence of *blaTEM*, *blaSHV*, *blaCTX-M* and Hatrongjit et al. (2018) for carbapenem resistance genes (*IMP*, *OXA-48 like*, *NDM* and *KPC*) and one of the colistin resistance gene *mcr-1* (Bauer et al., 1966; Bektaş et al., 2018; EUCAST, 2017a; EUCAST, 2017b; Hatrongjit et al., 2018) (Table 3 and Table 4). Colistin resistance was investigated using the PCR method including multiplex pcr with carbapenem resistance genes according to to determine the presence of the *mcr-1* gene (Hatrongjit et al., 2018).

Polymerase Chain Reaction (PCR) assays were employed to detect genes associated with extended-spectrum β-lactamases (ESBLs), carbapenemases, and colistin resistance. Target gene regions were amplified using specific primer sets, synthesized commercially, based on sequences previously reported by Bektaş et al. (2018) and Hatrongjit et al., 2018 respectively (Bektaş et al., 2018; Hatrongjit et al., 2018).

For the detection of ESBL genes (*blaTEM*, *blaSHV*, *blaCTX-M*), PCR reactions were carried out in a final volume of 25 µL, which included 5 µL of template DNA and 20 µL of PCR master mix. The PCR mix consisted of 12.5 µL of DreamTaq PCR Master Mix (2X) (Thermo Scientific, USA), 7.3 µL of DEPC-treated water, and 0.1 µL of each forward and reverse primer (100 pmol/µL). Amplification conditions were applied according to the protocol described by Bektaş et al., (2018).

Carbapenemase and colistin resistance genes were screened through multiplex PCR, following the methodology described by Hatrongjit et al., (2018). Each 15 µL reaction included 2 µL of extracted DNA and 13 µL of PCR master mix. This mix contained 8.8 µL of DreamTaq PCR Master Mix (2X), 2.2 µL of DEPC-treated water, and 0.2 µL of each forward and reverse primer (100 pmol/µL). Amplification conditions followed the protocol of Hatrongjit et al., (2018), as detailed in Table 3.

All PCR products, including those amplified for carbapenemase, *mcr-1*, and ESBL genes, were resolved by gel electrophoresis. A total of 10 µL of PCR product was mixed with 2 µL of 10X BlueJuice gel loading buffer (Thermo Scientific, USA) and loaded onto a 1.5% agarose gel (Prona) prepared in 1X Tris-Borate-EDTA (TBE) buffer. Electrophoresis was conducted, and DNA bands were visualized using a gel documentation system (EBOX CX5 TS EDGE, Vilber). A 100 bp DNA ladder (GeneRuler 100 bp, Thermo Scientific, USA) was used as a molecular weight marker.

In the study, reference strains of *Salmonella enterica* subsp. *enterica* serovar Enteritidis (ATCC® 13076™), *E. coli* (ATCC® 25922™), IMP: *E. coli* NCTC 13476, NDM: *K. pneumonia* NCTC 13443, KPC: *K. pneumonia* CCUG 56233, mcr-1: *E. coli* NCTC 13846, and CTX-M: *E. coli* CCUG 62975 were used as positive controls in conventional and molecular methods obtained from the Ministry of Agriculture and Forestry Giresun Food Control Laboratory and the Ministry of Health, General Directorate of Public Health, Microbiology Reference Laboratory. PCR master mix (without DNA) was used as the negative control in the PCR method.

Table 1. Genus-specific primers targeting the *16S rRNA* gene of *Salmonella enterica.*

Primers	Sequence	Target Gene	Base Pairs	Reference
16S rRNA-F	TGTTGTGGTTAATAACCGCA	*16S rRNA*	574 bp	Mir et al. (2015)
16S Rrna-R	CACAAATCCATCTCTGGA			

Table 2. *Salmonella 16S rRNA* gene's amplification conditions in PCR

PCR Steps	Cycles Conditions	Cycles numbers	References
Initial Denaturation	94°C, 2 min.	1 cycle	
Denaturation	94°C, 20 sec.		
Annealing	54°C, 20 sec.	30 cycles	Mir et al. (2015)
Extention	72°C, 30 sec.		
Final Extention	72°C, 2 min.	1 cyle	

Table 3. Carbapenem resistance and *mcr-1* genes' amplification condiditons in PCR

PCR Steps	Cycles Conditions	Cycles numbers	References
Initial Denaturation	95°C, 30 sec.	1 cycle	
Denaturation	95°C, 30 sec.		
Annealing	56°C, 30 sec.	30 cycles	Hatrongjit et al. (2018)
Extention	72°C, 45 sec.		
Final Extention	72°C, 5 min.	1 cyle	

RESULTS

A total of 34 *Salmonella* spp. were isolated using the ISO 6579-2017 method (ISO, 2017) (Figure 1) from stool samples and identified with biochemical tests (ISO, 2017; Quinn et. al., 2004) and genus-specific primers targeting the *16S rRNA* gene of *Salmonella enterica* (Mir et al., 2015) in PCR (Figure 2).

No extended-spectrum beta-lactamase activity was detected in 34 *Salmonella* spp. isolates using the disk diffusion test according to EUCAST (EUCAST, 2017a; EUCAST, 2017b), Jean et al. (2015) and PCR results.

According to the method specified in the EUCAST standard (EUCAST, 2017b), one *Salmonella* spp. isolate was identified as carbapenem-resistant, which had a meropenem zone <25mm and resistance to piperacillin/tazobactam according to EUCAST and CLSI guidelines, respectively (CLSI, 2020; EUCAST, 2017b). 33 of 34 *Salmonella* isolate no showed carbapenem resistance according to EUCAST guideline (EUCAST, 2017b).

Phenotypic analysis of resistance mechanisms was conducted in accordance with EUCAST guidelines (EUCAST, 2017b) and the method described by van Dijk et al. (2014). Resistance to temocillin was indicative of the presence of *OXA-48-like* genes. In one carbapenem-resistant *Salmonella* spp. isolate, the presence of a metallo-β-lactamase (MBL) mechanism was confirmed by enhanced synergy in the combined disk diffusion test using dipicolinic acid (DPA), supporting the presence of the *IMP* gene (EUCAST, 2017b; van Dijk et al., 2014).

Serotyping (ISO, 2017) were done to only this carbapenem-resistant one *Salmonella* spp. strain in Microbiology Department (Salmonella reference laboratory) in Ankara University Faculty of Veterinary Medicine, Türkiye. Because this strain is epidemiologically important as well as fo One Health principles. The carbapenem-resistant isolate was serologically typed as *S.* Infantis according to Kauffman-White scheme (ISO, 2017) by Salmonella referance laboratory.

According to the EUCAST procedure, an isolate phenotypically determined to be resistant to carbapenems and serotyped as *Salmonella* Infantis was found to carry the *IMP* and *OXA-48-like* carbapenem resistance genes based on PCR results (Figure 3). Carbapenem resistance genes were not detected by PCR in 33 of 34 *Salmonella* isolates; it was not detected phenotypically according to EUCAST procedure. Phenotypic results were found to be consistent with genotypic findings.

As a result of, carbapenem resistance was identified both phenotypically and genotypically in one isolate, which was serotyped as *Salmonella* Infantis. The colistin resistance gene *mcr-1* was not detected in 34 *Salmonella* isolates by PCR. All results were shown on Table 4.

Figure 1. Isolated *Salmonella* spp. On XLT_4 agar

Table 4. Results of isolation, identfication, carbapenem resistance and ESBL, *mcr-1* gene

Number of Stool	Isolated and Identified *Salmonella* spp.	Carbapenem resistant *Salmonella* spp. by phenotypic method	Carbapenem resistant *Salmonella* spp. by PCR	ESBL positive *Salmonella* spp. by phenotypic method and PCR
110	34 (30.9%)	1 (2.94%)	1 (2.94%) (*IMP* and *OXA-48-like* genes detected)	-

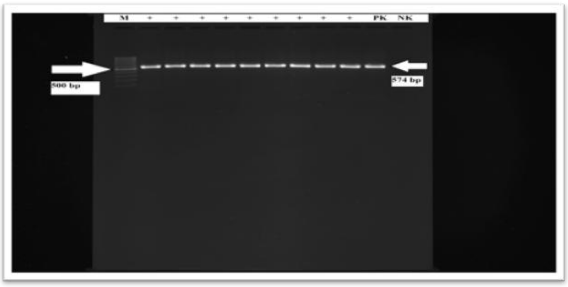

Figure 2. PCR results of *16S rRNA* gene of *Salmonella enterica*

M: Marker (100 bp DNA ladder (GeneRuler 100 bp, Thermo Scientific, USA), +: *16S rRNA* gene of *Salmonella enterica* positive strains, PK: Positive control, NK: Negative control.

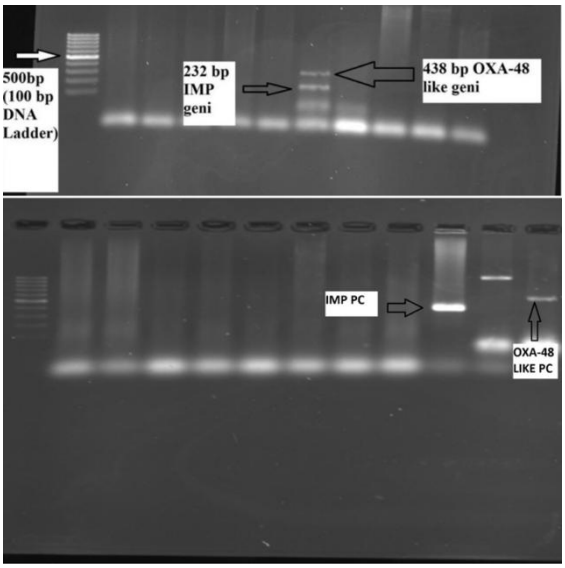

Figure 3. PCR results for carbapenemase resistance genes identified the presence of the *IMP* and *OXA-48-like* genes in *Salmonella* isolates.

M: Marker (100 bp DNA ladder (GeneRuler 100 bp, Thermo Scientific, USA), -: carbapenem resistance genes

DISCUSSION AND CONCLUSION

This study reports, for the first time, the presence of carbapenem resistance in *Salmonella* isolates obtained from chicken stool in Türkiye. Yıldız and Demirbilek (2024) have reported that they have found carbapenem resistant *S.* Enteritidis from dog isolates.

Dishan et al. (2024) reported that, in a study conducted in Türkiye, they isolated 112 *Salmonella enterica* strains from 293 chicken meat samples using the ISO 6579 method, and that all of these isolates were susceptible to meropenem.

Given the significance of Salmonella infections in poultry and their potential for zoonotic transmission through the food chain within the One Health approach, the identification of a carbapenem-resistant *Salmonella* isolate was deemed to be of considerable epidemiological importance.

The development of antimicrobial resistance (AMR) is associated with the inappropriate use of primary treatment drugs and the extensive application of antimicrobial compounds, along with the rising demand for animal-derived food products (Lozano-Villegas, 2024).

The development of antibiotic resistance in *Salmonella* spp. isolated from animal-derived food products predominantly occurs via two main pathways: co-resistance, in which a single genetic determinant confers resistance to multiple antimicrobial agents, and the simultaneous presence of distinct resistance genes targeting different antibiotic classes. Co-resistance facilitates the persistence and dissemination of *Salmonella* strains with resistance to a broad spectrum of antibiotics, thereby contributing to the emergence of multidrug-resistant (MDR) isolates, defined as those exhibiting resistance to three or more classes of antimicrobials. The proliferation of MDR *Salmonella* poses a serious public health risk, particularly due to their resistance to critically important antibiotics such as carbapenems, fluoroquinolones and third-generation cephalosporins (Oh et. al., 2025).

According to the 2024 EFSA (European Food Safety Authority) report, multidrug resistance is observed in *Salmonella* strains isolated from poultry meat in both developed and developing countries. In particular, the emergence of carbapenem-resistant *Salmonella* spp. isolates has been reported in 2021 and 2022 (EFSA, 2024).

Di Taranto et al. (2025) were declared that among the 128 *Salmonella* strains analyzed, 16 isolates (12.5%) were identified as extended-spectrum β-lactamase (ESBL) producers, all of which also displayed MDR profiles. These findings reinforce the role of chicken products as a significant reservoir of *Salmonella* spp. and emphasize that *S.* Infantis was the most frequently detected serotype, accounting for 85.93% of all isolates.

Dehdasti et al. (2024) were declared that they found NDM-1 gene in 6 of 39 *Salmonella* strains.

Kanaan et al. (2022) were showed that among the 20 *S.* Enteritidis isolates resistant to carbapenems, the most frequently detected carbapenemase gene was *blaIMP* (35.0%, n = 7), followed by *blaOXA−48−like* (25.0%, n = 5) and *blaNDM* (10.0%, n = 2). Notably, no isolates harbored the *blaKPC*or *blaVIM* genes.

Similar with Kanaan et al. (2022), in this study, *IMP* and *OXA-48-like* carbapenem resistance genes was detected in *S.* Infantis isolate.

In the report published by the European Food Safety Authority (EFSA) in 2017, *Salmonella Infantis* was reported as the fourth most common serotype in humans, and notably, it was identified as the most prevalent

serotype in poultry over the past five years, accounting for 33.6% of cases (Torun and Müştak, 2019).

In our country, studies conducted under the Salmonella Control Program initiated by the Ministry of Agriculture and Forestry in 2018 have shown that *S.* Infantis the most common *Salmonella* serotype in chicken isolates in Türkiye (Republic of Türkiye, Ministry of Agriculture and Ministry, 2018). Also Yapıcıer and Sareyyüpoğlu (2022) was reported that the most isolated seroype was *S.* Infantis in their study. The detection of carbapenem resistance in the *S.* Infantis serotype in this study is considered significant due to the prevalence of this serotype in our country. From a public health perspective, especially considering that carbapenem antibiotics are used for human treatment, it is believed that carbapenem antibiotics may not be effective in treating foodborne *S.* Infantis infections, and resistance may spread among bacteria.

This study reports, for the first time, the presence of carbapenem resistance in *Salmonella* isolates obtained from chicken stool and the *S.* Infantis serotype in Türkiye. In this study, carbapenem resistance was detected in one *Salmonella* isolate identified as *S.* Infantis. However, it was thought that this resistance may be part of the global increase in carbapenem resistance and multidrug resistance (MDR) observed in *Salmonella* spp..

Phenotypic results were found to be consistent with genotypic findings for carbapenem resistance. An isolate phenotypically determined to be resistant to carbapenems and serotyped as *S.* Infantis was found to carry the *IMP* and *OXA-48-like* carbapenem resistance genes based on PCR results. Carbapenem resistance genes were not detected by PCR in 33 of 34 *Salmonella* isolates; it was not detected phenotypically according to EUCAST procedure. In this study, this result demonstrated that the carbapenem resistance determined phenotypically was also genetically expressed.

The identification of carbapenem resistance genes via PCR in an isolate serotyped as *S. Infantis*, which exhibited phenotypic resistance based on the EUCAST guidelines, was considered epidemiologically important within the scope of the One Health concept, due to the possibility of horizontal gene transfer.

Carbapenemases are regarded as highly significant from an epidemiological perspective, especially when they reduce the effectiveness of carbapenem antibiotics such as imipenem, meropenem, ertapenem, and doripenem. These enzymes are particularly concerning due to their capacity to mediate resistance against nearly all β-lactam antibiotics and their ability to spread efficiently through horizontal gene transfer. Consequently, carbapenemase-producing organisms often exhibit multidrug resistance and are associated with infections that carry substantial mortality risks (EUCAST, 2017b). OXA-48-like carbapenemases are swiftly proliferating across Europe (Hopkins et al., 2019). Considering that *Salmonella* is most commonly transmitted to humans through poultry products as a foodborne pathogen, the detection of a carbapenem-resistant *Salmonella* isolate in this study is of particular significance within the One Health concept, especially given the critical role of carbapenem antibiotics in human medicine. It was considered that conducting both phenotypic and genotypic surveillance including all carbapenem genes for carbapenem resistance in bacteria could be beneficial. In this study, carbapenem resistance was detected in only one of the *Salmonella* spp. isolates and one province region of country; however, as the World Health Organization (WHO, 2017) has reported the global dissemination of carbapenem-resistant *Enterobacteriaceae* strains, further investigations are considered necessary for identified all carbapenem resistance genes and all regions of country. In future studies, plasmids or other mobile genetic elements could be identified for epidemiological typing, allowing the determination of clonal relationships and the potential risk of outbreaks. Moreover, it is considered important to perform both phenotypic and genotypic screenings for carbapenem resistance in isolates obtained from animals. Assessing the genetic similarities between carbapenem-resistant isolates from human and animal sources may provide valuable insights into their potential epidemiological linkage.

Particularly, as carbapenem-resistant isolates may cause infections in humans as foodborne pathogens, it is considered that the continuous monitoring of carbapenem resistance through the One Health approach is essential.

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Ethical Declaration

Stool, feces or litter collected from coop floor and clinical applications for diagnosis and treatment are not subject to ethics committee approval according to Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik 8-k, ethics committee permission is not required for this study.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept:Concept: O.B., Design: O.B., Data Collection or Processing: O.B., Analysis and Interpretation: O.B., Literature Search: O.B., Writing: O.B.

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REFERENCES

Abdel-Kader F, Hamza E, Abdel-Moein KA, Sabry MA. 2022. Retail chicken giblets contaminated with extended-spectrum cephalosporin-and carbapenem-resistant *Salmonella* enterica carrying blaCMY-2. Veterinary world, 15(5): 1297–1304.

Akgül M, Kul S, Öksüztepe G. 2021. Salmonellozis and foods of animal origin. Fırat University Veterinary Journal of health science, 35(2): 114–119.

Babacan O, Karadeniz H. 2019. Investigation of antibiotic susceptibility of *Salmonella* spp. strains' isolated from raw chicken meat. Journal of the turkish veterinary medical society, 90 (2): 105–114.

Bauer AW, Kirby WM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology, 45: 493- 496.

Bektaş A, Güdücüoğlu H, Gürsoy NC, Berktaş M, Gültepe MS, Parlak M, Otlu B, Takerekoğlu MS. 2018. Investigation of extended spectrum beta-lactamase (ESBL) genes in ESBL-producing Escherichia coli and Klebsiella pneumoniae strains. Flora the journal of ınfectious diseases and clinical microbiology, 23, 116-123.

Bénao SLCS, Métuor DA, Ouattara AK, Tiemtoré RYW, Ouédraogo N, Que B, Badini RO, Simpore J. 2024. Multidrug-resistant of Escherichia coli and *Salmonella* spp. strains in chicken feces ıntended for consumption in open spaces of Ouagadougou, Burkina Faso. Open journal of applied sciences, 14 (04): 881–892.

Clinical Laboratory Standards Institute (CLSI). 2020. Performance standards for antimicrobial susceptability testing. M-100, 30th. Ed. Wayne, PA 19087, USA.

Dehdashti S, Mohseni P, Ghanbarpour R, Aslani S, Moradiyan MS, Kalantar-Neyestanaki D. 2024. The emergence of carbapenem-resistance and New Delhi metallo-β-lactamase-1 (blaNDM-1) among *Salmonella* spp. in Kerman, Iran. Iranian journal of microbiology, 16(1):29–38.

Dishan A, Hizlisoy H, Onmaz NE, Yıldırım Y, Gönülalan Z, Al S. 2024. Comprehensive analysis of Salmonella in poultry meat and products in Türkiye: Prevalence, antibiotic susceptibility and genomic characterisation. International journal of food science and technology, 59(5): 3412-3422.

Di Taranto P, Petruzzi F, Normanno G, Pedarra C, Occhiochiuso G, Faleo S, Didonna A, Galante D, Pace L, Rondinone V, Trisolini C, Del Sambro L, Beverelli M, Catanzariti R, Caruso M, Palazzo L, Di Castri A, Parisi A. 2025. Prevalence and Antimicrobial Resistance of *Salmonella* Strains Isolated from Chicken Samples in Southern Italy. Microorganisms, 13(2).

European Committee on Antimicrobial Susceptibility Testing (EUCAST). 2017a. Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0. Available at: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_ Tables.pdf. (Accessed September 16, 2024).

European Committee on Antimicrobial Susceptibility Testing (EUCAST). 2017b. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.0. Available at https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf. (Accessed September 16, 2024).

European Food Safety Authority (EFSA). 2024. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2024. EFSA Journal, 22:e8583.

Hatrongjit R, Kerdsin A, Akeda Y, Hamada S. 2018. Detection of plasmid-mediated colistin-resistant and carbapenemresistant genes by multiplex PCR. MethodsX, 5: 532- 536.

Hopkins KL, Meunier D, Mustafa N, Pike R, Woodford N. 2019. Evaluation of temocillin and meropenem MICs as diagnostic markers for OXA-48-like carbapenemases. Journal of antimicrobial chemotherapy, 74, 3641–3643.

Hossain MJ, Attia Y, Ballah FM, Islam MS, Sobur MA, Islam MA, Ievy S, Rahman A, Nishiyama A, Islam MS, Hassan J, Rahman MT. 2021. Zoonotic Significance and Antimicrobial Resistance in *Salmonella* in Poultry in Bangladesh for the Period of 2011–2021. Zoonotic Diseases, 1(1), 3–24.

International Standard Organisation. 2017. ISO 6579: Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp.

Jean SS, Lee WS, Bai KJ, Lam C, Hsu CW, Yu KW, Liao CH, Chang FY, Ko WC, Wu JJ, Chen YH, Chen YS, Liu JW, Lu MC, Liu CY, Chen RJ, Hsueh PR. 2015. Relationship between the distribution of cefepime minimum inhibitory concentrations and detection of extended-spectrum β-lactamase production among clinically important Enterobacteriaceae isolates obtained from patients in intensive care units in Taiwan: results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) in 2007. Journal of Microbiology, Immunology and Infection, 48(1), 85-91.

Kanaan MHG, Khalil ZK, Khashan HT, Ghasemian A. 2022. Occurrence of virulence factors and carbapenemase genes in *Salmonella enterica* serovar Enteritidis isolated from chicken meat and egg samples in Iraq. BMC microbiology, 22(1), 1–8.

Kırkan Ş, Savaşan S, Parın U, Yüksel HT. 2017. Kanatlı yetiştiriciliğinde çoklu antibiyotik direnci ve risk yönetimi. Turkiye Klinikleri Journal of Veterinary Science Pharmacology and Toxicology -Special Topics, 3, 264-8

Kutu A. 2017. Isolatıon, Serotyping and investigation of antibiotic susceptibilities of Salmonella species in poultry. MSc Thesis. Adnan Menderes University, Health Science Institute, Department of Microbiology, Aydın province, Türkiye.

Lozano-Villegas KJ, Rondon-Barragan IS. 2024. Virulence and antimicrobial-resistant gene profiles of *Salmonella* spp. ısolates from chicken carcasses markets in Ibague City, Colombia. International journal of microbiology, 2024:4674138.

Mir IA, Kashyap SK, Maherchandani S. 2015. Isolation, serotype diversity and antibiogram of *Salmonella enterica* isolated from different species of poultry in India. Asian pacific journal of tropical biomedicine, 5 (7): 561-567.

Mondal AH, Khare K, Saxena P, Debnath P, Mukhopadhyay K, Yadav D. 2024. A Review on colistin resistance: An Antibiotic of Last Resort. Microorganisms. 12(4), 772.

Oh H, Choi Y, Lee J. 2025. Antibiotic-Resistant Salmonella in Animal Products Jeopardize Human Health. Food science of animal resources, 45(2), 409–428.

Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC. 2004. *Enterobacteriaceae.* in: Veterinary microbiology and microbial disease. Kundli: India Replica Press Ptv Ltd., pp. 106.

Republic of Türkiye, Ministry of Agriculture and Ministry. 2018. National Salmonella Control Programme. Available at: https://www.tarimorman.gov.tr/Konu/2083/Ulusal_Salmonella_Kontrol_Programi (Accessed May 20, 2025).

Salar MÖ, Yardımcı H, Diker KS. 2015. Bazı endüstriyel bitkilerin *Salmonella* serotipleri üzerindeki antimikrobiyel etkileri. Journal of the turkish veterinary medical society, 86: 9-18.

Sevük Akkaya NS, Ak S. 2023. *Salmonella* serovars and antibiotic resistance in chickens. Dicle university journal of faculty of veterinary medicine, 16 (2):81-86.

Şahan Ö, Aral EM, Aden MMA, Aksoy A, Yılmaz Ö, Jahed R, Akan M. 2016. Distribution and antibiotic resistance of Salmonella isolates from broiler enterprices in Turkey. Ankara üniversitesi veteriner fakültesi dergisi, 63, 1-6.

Temelli S, Kahya S, Eyigör A, Çarlı KT. 2012. Antibiotic resistance phenotypes of Salmonella isolates of

broiler meat and chicken origin. Ankara üniversitesi veteriner fakültesi dergisi, 5, 107-114.

Torun E, Müştak HK. 2019. Isolation and characterization of *Salmonella* Infantis phages from poultry faces and environmental samples. Etlik veteriner mikrobiyoloji dergisi, 30(2): 149–157.

van Dijk K, Voets GM, Scharringa J, Voskuil S, Fluit AC, Rottier WC, Stuart JC. 2014. A disc diffusion assay for detection of class A, B and OXA-48 carbapenemases in Enterobacteriaceae using phenyl boronic acid, dipicolinic acid and temocillin. Clinical microbiology and ınfection, 20(4): 345-9.

Waktole H, Ayele Y, Ayalkibet Y, Teshome T, Muluneh T, Ayane S, Antonissen G. 2024. Prevalence, molecular detection, and antimicrobial resistance of Salmonella isolates from poultry farms across Central Ethiopia: A cross-sectional study in Urban and Peri-Urban areas. Microorganisms, 12 (4).

World Health Organisation. 2017. Available at: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed (Accessed July 28, 2025).

Yapıcıer ÖŞ, Sareyyüpoğlu B. 2022. Prevalence and rapid identification of Salmonella Infantis in broiler production in Turkey. Ankara üniversitesi veteriner fakültesi dergisi, 69 (1), 1-8.

Yıldırım M, Baş B, Babacan O. 2022. Salmonella enfeksiyonları, in: Prof. Dr. Ender Yarsan (Eds), Kanatlı Hekimliği. Güneş Tıp Kitabevleri Ltd. Şti, Ankara, Türkiye, pp.393- 404.

Yildiz M, Demirbilek SK. 2024. Investigation of prevalence and antimicrobial resistance of Salmonella in pet dogs and cats in Turkey. Veterinary medicine and science, 10(4): 1–12.