



Serotype Distribution and Antimicrobial Resistance Profile of *Salmonella* Isolated from Traditional Chicken Doner Kebabs in Türkiye

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ABSTRACT

This study aimed to investigate the presence of *Salmonella* spp. in chicken doner samples, both ready for consumption and sold in packages in a modified atmosphere, using classical culture technique and the MALDI-TOF method by determining the antibiotic resistance profile structure through the disc diffusion method by determining the serotypes of the isolates through mPCR. Between September and November 2019, 150 ready-to-eat chicken doner samples (75 of them ready to consume, the other 75 packed in a modified atmosphere) were analyzed. It was found that 23 of the 150 (15.3%) samples analyzed were contaminated with *Salmonella* spp. 73 isolates were obtained from 23 positive samples. Of these 73 isolates, 33 were found to be *Salmonella* spp. positive. In serotyping, all 33 isolates were determined to be *S. infantis*. In the antibiotic resistance profile, 30 of the isolates were resistant to chloramphenicol, 24 to gentamicin, 21 to ampicillin, 20 to trimethoprim-sulfamethoxazole and ciprofloxacin, 18 to tetracycline and amoxicillin-clavulanic acid, and 13 to ceftiofloxacin. As a result, the isolation of *Salmonella* spp. from the samples and their resistance to different antibiotics were evaluated as potential sources of problems in the context of foodborne infectious diseases.

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Introduction

Due to socio-economic and cultural interactions, dietary habits have turned into a lifestyle defined as fast food, in which the foods that are prepared and consumed in a short time have come to the fore (Panozzo et al., 2015). Among these foods, doner, which is an economical meat product with high nutritional value, has been reported to have a history of almost 4000 years in some sources, and the term doner was first coined by "Iskender Bey" 150 years ago (Al-Shadefat and Gürbüz, 2016). Doner is known by different names such as "donair, doner, gyros, donakebab, donnakebab, shawarma, chawarma" in various parts of the world (Kayisoglu et al., 2003). Kebab doner is one of the fastest-growing sectors in the fast-food market in some regions of Europe, including the UK (Meldrum et al., 2009). Doner has seemingly become the favorite food for almost 30 per cent of Italians under 35 (Panozzo et al., 2015). According to 2015 statistics, doner production in Türkiye is approximately 500 tons. In addition to that, it is reported that there are 25.000 doner kebab sales points in Germany and more than 50.000 in all European countries

(Ozsaraç et al., 2019). In the Turkish Food Codex (TGK, 2019), doner is defined as a "meat product prepared by adding flavorings and other food ingredients of bovine/ovine carcass meat or poultry carcass meat, formed in a cylindrical shape on a doner skewer, prepared by rotating a mixture of red or poultry meat ready to be cooked horizontally or vertically".

Poultry meat is one of the most preferred animal foods all over the world, as it is a good source of protein, amino acids, minerals, and vitamins necessary for the mental and physical development of people. According to current data (BESD-BİR, 2018), in 2018, the average poultry meat consumption per capita in the United States was 49.8 kg, 24.5 kg in European Union countries, and 21.9 kg in Türkiye. It has been reported that a portion of doner can meet 40% of the total daily energy requirements and 90% of the protein needs of people on average, depending on the type of raw material used and gender (Panozzo et al., 2015). Also, poultry meat, which plays an important role in meeting protein needs, creates a suitable breeding

environment for microorganisms with the nutrients it contains. For example, in the production of doner, there are many factors which introduce a greater risk environment for bacterial infections, such as the application of insufficient and ineffective cooking temperatures, slicing the doner too thick, and the addition of spices or certain additives (Bostan et al., 2011).

Salmonella is one of the most common causes of foodborne illnesses worldwide. Poultry is one of the foods responsible for *Salmonella* infection, and the rate of foodborne *Salmonella* infections is recorded as 47%. Due to the high morbidity and mortality prevalence of *Salmonella* serotypes, the examination of poultry meat for *Salmonella* is crucial for public health (Kimura et al., 2004; Park et al., 2017; Marcus et al., 2002; Tonbak et al., 2017). Antibiotics are the essential drugs used in bacterial infections in both animals and humans. Gram-negative and gram-positive bacteria are used in all treatments (Krupodorova et al., 2022). However, their broad-spectrum use causes antimicrobial resistance to bacterial pathogens. This resistance causes infections that are difficult to treat with traditional antimicrobials, which poses a worldwide problem in addition to high morbidity and mortality (Sevindik et al., 2017; Singh et al., 2017; McMillan et al., 2019; Mohammed et al., 2022).

Within the scope of this study; i) *Salmonella* spp. was isolated using classical culture, ii) identification of suspected isolates was performed by MALDI-TOF method, iii) confirmation of the isolates was completed using the PCR method, iv) *Salmonella* spp. serotypes were determined by mPCR, and v) the resistance of the isolates to antibiotics was determined through disk diffusion testing.

Material and Methods

A total of 75 MAP (modified atmosphere packed) chicken doner samples were taken regularly each month for 3 months between September and November 2019, and 75 chicken doner samples sold daily in Samsun/Türkiye were immediately analyzed for the presence of *Salmonella* spp. after they were brought to the laboratory under the cold chain. With regards to the selection of samples, the 75 MAP chicken doner samples were collected from 5 different companies, while the other 75 chicken doner kebab samples sold daily by restaurants in Samsun were collected from 51 different sales points.

Salmonella spp. Isolation Classical Culture Technique

The methods reported by ISO 6579 - The International Organization for Standardization (ISO-6579, 2002), FDA-Food and Drug Administration (2003), and Lee et al. (2015) were used for the isolation of *Salmonella* spp. after weighing 25 g of each sample in filtered sterile plastic bags under aseptic conditions and adding 225 mL of buffered peptone water (TPS) (Merck, 1.7228) to each sample. The mixture was homogenized in the stomacher for 2-3 minutes, and aerobes were incubated for 24 hours at 37°C for pre-enrichment conditions. Following the pre-enrichment process, for selective enrichment, 0.1 mL of pre-enrichment homogenate was added to the tubes containing 10 ml Rappaport-Vassiliadis Broth (RVB)

(Merck,1.07700) and incubated at 43°C for 24 hours, respectively. After selective enrichment, a loopful of homogenate was taken from the tubes. Brilliant green Phenol-red Lactose Sucrose Agar (BPLS) (Merck, 1.07237) was planted with the scratching method, and the plates were incubated at 37°C for 24-48 hours. 3-5 of the typical pink-red colonies grown on BPLS agar with smooth edges and color were selected for Tryptic Soy Agar.

MALDI-TOF Method in *Salmonella* spp. Identification

Suspected *Salmonella* isolates were subcultured on Tryptic Soy Agar (Merck, 1.05458) and identified by VITEK MS (MALDI-TOF). *E. coli* ATCC 8739 was used as the reference strain (Sparbier et al., 2012).

Verification of *Salmonella* spp. Isolates by PCR.

Following the optimization of the PCR conditions, PCR was performed using the primer pairs (F: 5'-TTA TTA GGA TCG CGC CAG GC-3', R: 5'-AAA GAA TAA CCG TTG TTC AC-3') that make up the *oriC* gene (163 bp) sequence under the protocol proposed by (Widjoatmodjo et al., 1991) and (Fluit et al., 1993).

Genomic DNA Extraction

Once the isolates were identified, DNA extraction was performed according to the boiling method. Accordingly, the isolates were incubated in Brain Heart Infusion broth (BHI) (Merck, 1.10493) for 24 hours at 37°C and a 1 mL aliquot was extracted, transferred to sterile eppendorf tubes, and centrifuged at 10.000×g for 5 minutes. Then, the supernatant was discarded, and 500 µL PBS was added and kept in a 95°C water bath for 10 minutes. Then it was centrifuged at 10.000×g for 5 minutes (Hettich Universal R320, Germany) and preserved at -20°C until PCR was performed as a supernatant template.

PCR Amplification and Electrophoresis

The PCR mix for the *oriC* gene was prepared in a total volume of 50 µL of 1X PCR Buffer, 1.5 mM MgCl₂, 100 µM dNTP, 2 µ Taq-Polymerase (Sigma D4545), 0.5 µM, and 10 µL DNA from each primer. *oriC* gene amplification in a Thermal Cycler (Bio-Rad MJ mini-Gradient CA-USA) was carried out under the following conditions: the first denaturation at 94°C for 5 min for 35 cycles; denaturation at 94°C for 1 min; primer ligation at 53°C for 1 second, a primary extension at 72°C for 1 min, and the final elongation was performed at 72°C for 10 minutes (6, 31). The electrophoresis process of the amplicons obtained was carried out in 2% agarose at an 80-volt current. Electrophoresis was performed in a Bio-Rad PowerPac Basic Power Supply (CA - USA) and a Bio-Rad Wide Mini Sub-Cell GT Cell (CA - USA) electrophoresis tank. Upon completion of electrophoresis, the *oriC* gene was visualized at 163 bp using a UV-transilluminator.

Serotyping of *Salmonella* spp. Isolates by Multiplex PCR

The isolates confirmed as *Salmonella* spp. by PCR on the *oriC* gene were serotyped by multiplex PCR (mPCR) using primer sets SET1, SET2, and SET3. Sero grouping primer set was used in SET1 mPCR, Phase-1 primer set

was used in SET2 mPCR, and Phase-2 primer set was used in SET3 mPCR.

SET 1 mPCR

Multiplex PCR was applied using selected primers for the gene regions encoding the "O" somatic antigens [O:B (O:4), O:C1 (O:7), O:C2-C3 (O:8), O:D (O:9, O:9,46, O:9,46,27), DNA amplification, 5 min pre-denaturation at 94°C, and 30 cycles at 94°C for 40 s, 59°C for 20 s, 72°C for 20 s, and 72°C for 7 min with final chain extension were carried out. Electrophoresis was performed on agarose gel (%1 w/v) with ethidium bromide (10 mg /ml), Bio-Rad PowerPac Basic Power Supply (CA-USA) with 50 bp DNA marker, and Bio-Rad Wide Mini Sub-Cell GT Cell (CA-USA) electrophoresis tank and examined using UV-transilluminator (Herrera-leon et al., 2007).

SET 2 mPCR

Specific primers were used for H: i, H: z10, H: b, H: e, h, H: 1, v, H: r, H: G complex flagellar antigens. Since the second phase of *Salmonella enterica subsp enterica* serovar Enteritidis (*Salmonella* Enteritidis) was not exhibited, *sdfl* primers were used to amplify this serovar specifically. DNA amplification in multiplex PCR application was performed at 95°C for 5 min pre-denaturation and 30 cycles at 95°C for 40 seconds, 59°C for 20 s, 72°C for 20 s, and 72°C for 7 minutes for some time with the final chain elongation. Electrophoresis was performed on agarose gel (%1 w/v) with ethidium bromide (10 mg /mL), a Bio-Rad PowerPac Basic Power Supply (CA-USA) with 50 bp DNA marker, and a Bio-Rad Wide Mini Sub-Cell GT Cell (CA-USA) electrophoresis tank and examined using a UV-transilluminator (Herrera-leon et al., 2004).

SET 3 mPCR

Specific primer sequences and sense-60 and antisense-Rw primer sequences were used for *fljB* genes (H1,2 ; H:1,5 ; H:1,6 ; H:1,7 ; H:l,w ; H:e,n,x and H:e,n,z15), encoding DNA amplification in multiplex PCR application was performed at 95°C for 5 min pre-denaturation and 30 cycles at 95°C for 40 s, 59°C for 20 s, 72°C for 20 s and 72°C for 7 minutes for some time with the final chain elongation. Electrophoresis was performed on agarose gel (%1 w/v) with ethidium bromide (10 mg /mL), a Bio-Rad PowerPac Basic Power Supply (CA-USA) with 50 bp DNA marker, and a Bio-Rad Wide Mini Sub-Cell GT Cell (CA-USA) electrophoresis tank and examined using a UV-transilluminator (Echeita et al., 2002).

Verification of *Salmonella Infantis* isolates by PCR.

After all the molecular serotyping procedures, all isolates were determined as *S. Infantis*, and a single PCR test was applied to the isolates for confirmation. For this purpose, primary sequences belonging to *fljB* (F: AAC AAC GAC AGC TTA TGC CG; R: CCA CCT GCG CCA ACG CT), gene-specific to *S. Infantis* serotype, were used (Kardos et al., 2007). In the amplification of the PCR process, first, denaturation for 1 minute at 95°C with 35 cycles, denaturation at 95°C for 30 seconds, primer binding at 57°C for 40 seconds, a primer for 30 seconds at 72°C extensions and final extensions parameters at 72°C for 5 minutes were used. The electrophoresis process of the

amplicons obtained was carried out in 2% agarose at 80-volt current, in a Bio- Rad PowerPac Basic Power Supply (CA-USA) and Bio-Rad Wide Mini Sub- Cell GT Cell (CA-USA) electrophoresis tank. Upon completion of electrophoresis, the *fljB* gene was imaged at 727 bp using the UV-transilluminator.

Antibiotic Resistance Tests

Antibiotic resistance of the obtained isolates was determined using the disk diffusion method in Mueller Hinton agar based on the methods reported by EUCAST-European Committee on Antimicrobial Susceptibility Testing/2018 (2018). Antibiotic resistance properties of *Salmonella* spp. isolates obtained in the study were performed on amoxicillin-clavulanic acid, trimethoprim/sulfamethoxazole, ciprofloxacin, cefoxitin, chloramphenicol, tetracycline, ampicillin, and gentamicin discs.

Results

Within the framework of analysis findings in the study; 23 (15.3%) of a total of 150 samples were found to be contaminated with *Salmonella* spp. When the distribution of analysis findings according to samples was examined, a total of 75 samples from 5 different companies were analyzed from MAP (modified atmosphere packaged) chicken doner samples, and 12 (16%) of them were found to be positive in terms of the agent. Of the 75 MAP chicken doner samples from 5 different companies, a total of 11 (16%) were found to be positive in terms of the agent. Concerning the samples of chicken doner kebab sold daily at enterprises in Samsun, of the 75 samples (which were collected from 51 different sales points: 1 sample each from 32 different establishments, 2 samples from 17 different establishments at different times, and 3 samples from 3 different establishments at different times), 11 samples (14.6%) were determined as positive for *Salmonella* spp. A total of 73 suspicious isolates were collected from chicken doner samples, and 33 of them were *Salmonella* spp. (Table 1). Of the 33 isolates, 18 (54.5%) were obtained from MAP packed chicken doner kebab samples, and 15 (45.5%) were obtained from ready-to-eat. In the statistical evaluation of *Salmonella* incidence, no significant difference was found between MAP packed chicken doner kebab samples and ready-to-eat samples (16% and 14.6%, P>0.05).

In serotyping, all 33 isolates (100%) were determined to be *Salmonella enterica* serotype Infantis. This result is shown in Table 2.

In our study, the antibiotic resistance profile analysis showed bacterias were found to be resistant as follows: 15 of 33 *S. Infantis* isolates were resistant to amoxicillin-clavulanic acid, 10 to ampicillin, 2 to gentamicin, 12 to trimethoprim/sulfamethoxazole, 15 to tetracycline, 3 to chloramphenicol, 18 to cefoxitin, and 7 to ciprofloxacin (Tables 3-4). Multiple resistance profiles were not detected in any of the isolates.

Discussion

Differences between studies, slaughterhouse cleanliness, cross-contamination of items, differences in tests used, bacterial load in the product, and seasonal

variation are thought to be strong reasons for the prevalence of *Salmonella*. It has been reported that as the population increases in the poultry houses or during the transportation of the chickens, the pathogen load may increase due to stress, and consequently, the bacterial load in the final product may change due to cross-contamination between carcasses (Izat et al., 1989).

Cooking temperatures in chicken doner samples are important in terms of *Salmonella* contamination. In the studies, it was determined that *Salmonella senftenberg* is the serotype that exhibits natural heat resistance. Among the serotypes that cause foodborne illness, *S. enteritidis* has often been shown to be the most heat-resistant serotype (Doyle et al., 2000; Ng et al., 1996).

El-Shdefat and Gürbüz (2016) has determined that the average internal temperature of chicken doner kebabs, which are offered for ready-to-eat before cooking, is 6.4-7.6°C, and this temperature is between 58.7-62.0°C at the time of sampling.

When the literature was reviewed, a limited number of studies reported the presence of *Salmonella* spp. in chicken doner kebabs, serotyping, and antibiotic resistance.

Isolates collected from these samples were reported to be 100% resistant to oxacillin, teicoplanin, clindamycin, vancomycin, and erythromycin (Omurtag et al., 2012), it was reported that only one sample had *Salmonella* spp. in chicken doner samples bought from 71 different sales points in Vienna.

Table 1. *Salmonella* spp. incidence of modified atmosphere packed and ready for consumption chicken doner.

Sample	Number of Samples	<i>Salmonella</i> spp. NPS	NIA	<i>Salmonellas</i> pp. PNI
Modified atmosphere packed	75	12	33	18
Ready for consumption	75	11	40	15
Total	150	23	73	33

NPS: Number of Positive Samples, PNI: Positive Number of Isolates, NIA: Number of Isolates Analyzed

Table 2. *Salmonella* species serotype type of chicken doner.

Sample Origin and Number	Classical Culture + MALDI-TOF	PCR (oriC gen)	Serotype
	<i>Salmonella</i> spp. Positive Number of Isolates	<i>Salmonella</i> spp. Positive Number of Isolates	<i>Salmonella enterica</i> serotype Infantis
Modified atmosphere packed (n:75)	18	18	18
Ready for consumption (n:75)	15	15	15
Total (n:150)	33	33	33

Table 3. Antibiotic resistance profile of *Salmonella* serotypes.

Antibiotic	<i>Salmonella enterica</i> serotype Infantis (n: 33)		
	Susceptible	Intermediate	Resistant
Amoxicillin-Clavulanic Acid (20 µg-10 µg/mL)	15	0	18
Trimethoprim-Sulfamethoxazole (1.25 µg-23.75 µg/mL)	12	1	20
Ciprofloxacin (5 µg/mL)	7	6	20
Cefoxitin (30 µg/mL)	18	0	13
Chloramphenicol (30 µg/mL)	3	0	30
Tetracycline (30 µg/mL)	15	0	18
Ampicillin (10 µg/mL)	10	2	21
Gentamicin (10 µg/mL)	2	7	24

Table 4. Antibiotic resistance profile of *Salmonella* serotypes by sample origin

Antibiotic Resistance (%)	Sample Origin / Number of Isolates	
	Ready for consumption (n:15)	Modified atmosphere packed (n:18)
	<i>Salmonella</i> serotype	<i>Salmonella</i> serotype
	<i>Salmonella enterica</i> serotype Infantis	<i>Salmonella enterica</i> serotype Infantis
Amoxicillin-Clavulanic Acid (20 µg-10 µg/mL)	9 (60%)	6 (33.3%)
Trimethoprim-Sulfamethoxazole (1.25 µg-23.75 µg/mL)	1 (6.6%)	11 (61.1%)
Ciprofloxacin (5 µg/mL)	0 (0%)	7 (38.8%)
Cefoxitin (30 µg/mL)	9 (60%)	9 (50%)
Chloramphenicol (30 µg/mL)	1 (6.6%)	2 (11.1%)
Tetracycline (30 µg/mL)	2 (13.3%)	13 (72.2%)
Ampicillin (10 µg/mL)	7 (46.6%)	3 (16.6%)
Gentamicin (10 µg/mL)	1 (6.6%)	1 (5.5%)

Regalado-Pineda et al. (2020) conducted a study by collecting 1160 chicken meat samples from January 2016 to December 2018 from five markets and five supermarkets in Mexico. As a result of this study, they detected 18.1% *Salmonella enterica* contamination in 210 samples and also reported that this rate increased from 13.7% to 27.1% from 2016 to 2018. Lytoun et al. (2020) who purchased 80 marinated chicken products from 40 different markets for three months from February to April in Athens, Greece reported that they detected *Salmonella* spp. in nine samples (11.25%) in their study. Perin et al. (2020), who conducted a study with 300 samples including wings, breast, thigh meat, and fried chicken collected from 35 facilities from August 2015 to February 2016 in the Parana state of Brazil, detected *Salmonella* spp. in 95 samples. They reported that 42 isolates were Typhimurium, and 38 isolates were Heidelberg. Finazzi et al. (2019) analyzed fresh chicken meat from Poland in cold storage in Italy, and in a study of 156 samples, 72 samples were found to be contaminated with *Salmonella* spp. Of the 222 isolates obtained from 72 samples, 149 were determined as Infantis, 42 as Enteritidis, 26 as Newport, 4 as Kentucky and 1 as Mbandaka. Wang et al. (2020) stated that they detected *Salmonella* spp. in 103 of 4225 poultry samples taken from diseased poultry houses between 2014-2018. They found that 55 of these isolates were Enteritidis, and 36 were Typhimurium and obtained the serotypes Apeyeme, Anatum, Senftenberg, Kottbus, Montevideo, and Pullorum. Kulasooriya et al. (2019) studied 124 samples of chilled, frozen, and cooked chicken meat as well as sausages and meatballs made from chicken meat between July and October 2014 in Kandy, Sri Lanka, and isolated *Salmonella* spp. in nine samples. Also, they did not detect *Salmonella* spp. in cooked chicken meat.

Although the use of antibiotics in poultry breeding has been preserved, the isolates were found to be resistant to antibiotics in the analyses conducted on the condition that the animals were not exposed to any antibiotic drug other than the disease during the breeding. It is thought that this may be the result of environmental factors, feed and water used for feeding animals and the constant presence of antibiotics in the business flora. Perin et al. (2020) reported that 100% of the 95 *Salmonella* spp. isolates obtained from various chicken meats in Brazil were resistant to chloramphenicol, 94% to tetracycline, 87% to ampicillin, 84% to amoxicillin-clavulanic acid, and % 76 to ciprofloxacin. Tirziu et al. (2020) found that 11 of the 12 *Salmonella* spp. isolates they obtained from chicken meat in Romania were resistant to ciprofloxacin and tetracycline, 5 to chloramphenicol, and 2 to ampicillin. Sasaki et al. (2021) reported that 100% of the 114 *Salmonella* spp. isolates they obtained from local and imported chicken meats in Iraq were resistant to ampicillin, 80% to tetracycline, 10% to gentamicin, and 30% to ciprofloxacin. Abraham et al. (2019) reported that 11.3% of *Salmonella* spp. isolates obtained from chicken meat in Australia were resistant to Cefoxitin, 3.8% to ampicillin and amoxicillin-clavulanic acid, and 1.9% to trimethoprim-sulfamethoxazole. According to the 2018 report of the National Salmonella Control Program in Türkiye (USKP, 2019), it has been reported that *Salmonella* isolates isolated from broiler, Türkiye,

breeding, and slaughterhouses are resistant to 10 antibiotics (streptomycin, ampicillin, cefotaxime, chloramphenicol, tetracycline, nalidixic acid, gentamicin, sulphamethoxazole, ciprofloxacin, trimethoprim). These isolates were 93.1% resistant to sulfamethoxazole, 87.2% to nalidixic acid, 18.5% to ampicillin, 33.5% to streptomycin, and 29.4% to tetracycline. It was reported that 32.2% of them were resistant to trimethoprim, 7.7% to chloramphenicol, and 7.1% to ciprofloxacin, and the lowest resistance was cefotaxime with 0.9%.

Conclusion

With these results, attention should be drawn to the cooking temperatures of doner kebabs sold in the open, hygiene rules of the staff working in restaurants, and the cleaning of the tools and equipment used while preparing doner. Also, it is necessary to comply with HACCP rules and to keep the hygiene of the facilities and equipment under constant control in the facilities producing doner packed in modified atmosphere. Since *Salmonella* spp. can also be transmitted by cross-contamination, facilities that breed chicken should be checked for hygiene, and their staff should have training on hygiene rules. To prevent the development of antibiotic-resistant *Salmonella* spp., unnecessary and inappropriate antibiotics should not be used in chickens raised for use in the food industry. In addition, antibiotics should be used under veterinary control, and legislation should be followed.

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