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Extended Spectrum Beta-Lactamase (ESβL), AmpC and carbapenemase activities and colistin resistance of *Salmonella* spp. isolated from food poisoning cases in Turkey

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Abstract: This study aims at detecting antimicrobial resistance properties including extended spectrum beta-lactamase (ESBL), AmpC, carbapenemase activities (imipenem, meropenem and ertapenem), and colistin resistance of isolated and serotyped *Salmonella* spp. strains from various foods (mostly chicken and chicken products) that cause food poisoning. The ISO 6579-3 (Kaufmann-White scheme) method was used for serotyping of *Salmonella* spp. and for detection of antimicrobial resistance with Minimum Inhibitory Concentration (MIC) as reported by EURL-AR. Inoculums were separated into EUVSEC and EUVSEC2 panels using the Sensititre AIM Automated Inoculation Delivery System. The results were monitored in a semiautomatic vision reader and evaluated according to EUCAST. Resistance to 14 different antibiotics of 34 serotyped *Salmonella* spp. strains were examined in the first panel in this study. Most of these strains were found to be resistant to ciprofloxacin, colistin, nalidixic acid, sulfonamides, tetracycline, tygecycline, and trimethoprim. Nine of these agents (26.4%) were determined as single-drug resistant and 20 of them (55.8%) were determined as multidrug-resistant. Only 2 strains were determined to be resistant to cefotaxime and ceftazidime; however, ESBL activity was not observed in the second stage of the analysis, in which EUVSEC2 panels were used. All the strains for carbapenemase activities were determined as sensitive to imipenem, meropenem, and ertapenem. Also, all the strains for AmpC activities were found to be sensitive to cefoxitin. The same resistance properties of the isolates were detected against nalidixic acid, ciprofloxacin, and tetracycline. Colistin resistance was detected as 44.1%. *Salmonella* spp. strains isolated from foods that caused food poisoning were determined as multidrug-resistant to antibiotics at a high rate. This study is the first one in Turkey that has evaluated ESBL, AmpC, carbapenemase activity, and colistin resistance of *Salmonella* spp., which was isolated from the food poisoning cases, by using MIC test (recommended by EURL-AR). In Turkey, antibiotics use should be avoided especially in chicken farms in order to prevent the increasing multidrug resistance of *Salmonella* spp.

Key words: *Salmonella* spp., MIC, ESBL, AmpC, carbapenemase, colistin

1. Introduction

Detection of antimicrobial resistance is a global phenomenon that requires the formation of antibiotic-resistant pathogens that cause significant infections in the clinic and the development of new therapeutic strategies. Antimicrobial resistant bacteria cause major health-disrupting diseases. The development of antimicrobial resistance is a result of approaches involving the intensive use of antibiotics in animals. Drug-resistant bacteria are often detected from various environmental samples, farms, and retail meat products [1].

Many factors such as human travel to other countries, international trade in food, animal movements, agricultural systems, livestock, and some types of animal primary production cause resistant clones to spread all over the world. Since 2003, the United States (U.S.) Food and Drug Administration (FDA) has reported that antimicrobial resistance seen in *Salmonella* spp. and other bacteria species has been a global threat and a growing public health problem. The emergence and spread of resistant clones complicates the use of antimicrobials in humans and animals in the US. According to the National Antimicrobial Resistance Monitoring System (NARMS), it has been reported that AMR bacteria can pass from animals to humans, thus decreasing the effectiveness of antimicrobial drugs used to treat humans, which is a common phenomenon [2].

By the Commission Decision 2013/652/EU, the European Union (EU) has developed a monitoring program of antimicrobial resistance in bacteria isolated from food and food-producing animals in all member states. Under this legislation, monitoring of antimicrobial resistance began in 2014. AMR data are collected from targeted food and food-producing animals and meat,

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especially from different poultry. The intensive use of antibiotics against zoonotic pathogenic microorganisms for protective and therapeutic purposes in animals in veterinary medicine and the use of growth promoters in animal feed may lead to the rise of resistant bacteria. It is noted that many foods, especially poultry meat and its products, are the most important sources of *Salmonella* spp. infections in humans. Many health problems have been reported in recent years, especially due to the emergence of highly resistant pathogenic bacteria species including *Salmonella* spp. in foods [3,4].

Salmonella Infantis is the most commonly reported serovar in broiler flocks and broiler meats that has MDR (multidrug resistance). Some clones are spread between chickens and humans, and *S.* Infantis isolates are reported to be very common in chicken meat industry in many EU member states. Resistant strains create a public health problem by passing from animals to humans. MDR *S.* Infantis clones are also frequently detected in chickens, slaughterhouses, retail meats, and humans [5].

Another study was performed on *Salmonella* strains isolated from broilers with predominant serotype *S.* Infantis and high antibiotic resistance rates in Turkey [6].

Salmonella enterica with multidrug resistance has been recognized as a high priority pathogen by the World Health Organization (WHO). In a study,– 264 *Salmonella enterica* isolates were collected over 16 years (2000–2016) from poultry and pork production chains and *S.* Infantis was reported to cause a clonal spread between food sources and humans and to have international lineages and permanently conserved genomes in the food industry [7].

A study in Iran revealed a high resistance to commonly used antibiotics for poultry that constitutes a threat to public health. Also, food-borne diseases from nontyphoid *Salmonella* spp. have been reported quite widely in the world. In this study, it was reported that the most common serotype isolated was *S.* Infantis (79.5%), and these strains could be transmitted directly to humans through food or could transmit resistant genes to humans. For these reasons, it has been reported that limiting antibiotic use in humans and animals and selecting appropriate drugs by performing antibiotic susceptibility tests may reduce the spread of resistant strains [8].

Colistin is widely used in the production of foods of animal origin, and as a result, genes that cause colistin resistance are passed from foods to the flora of humans via plasmids. It is reported that controlling the use of colistin is highly important to reduce the proliferation and spread of colistin-resistant bacteria. A limited number of effective antibiotics are reported to have caused the use of colistin, an ancient antibiotic. Colistin is widely used in animal production in many countries for therapeutic, prophylactic, and growth purposes. Resistant bacteria are reported to be spreading through contact or food chain as a result of low doses of long-term antibiotic use in animals [9].

According to EFSA (2014), it was reported for *Salmonella* and *Escherichia coli* (*E. coli*) that resistance to ampicillin, fluoroquinolones, tetracyclines, and sulfonamides is common in meat isolates, while resistance to third generation cephalosporins is rare. This study, organized by EFSA, was used for the first time to monitor ESBL/AmpC/carbapenemase production in *Salmonella* and *E. coli*. At the end of this study, it was reported that ESBL/AmpC production was low and carbapenemase production was not detected, and colistin resistance was low in *Salmonella* and *E. coli* isolated from poultry and meat [10].

A complex situation has been reported in broiler chickens, meats, and humans due to the spread of new *Salmonella* Infantis strains with multidrug resistance and producing ESBL [11].

Spot mutations in DNA gyrase and topoisomerase IV genes have been reported to be associated with quinolone resistance in *Enterobacteriaceae* and *Salmonella* spp. These mutations reduce resistance to nalidixic acid and susceptibility to fluoroquinolones such as ciprofloxacin [12]. Fluoroquinolone antibiotics have been widely used in the treatment of bacterial diseases and it has been reported that quinolone resistance has become a serious concern in recent years due to the increase in antimicrobials and their inappropriate use [13].

The aim of this study was to determine the antimicrobial resistance properties of *Salmonella* spp. isolated and serotyped from food poisoning cases by the MIC (Minimum Inhibitory Concentration) test recommended by the European Union Reference Laboratory-Antimicrobial Resistance (EURL-AR) and the presence of ESBL, AmpC, and carbapenemase activities (imipenem, meropenem, and ertapenem) of *Salmonella* spp. isolates. Colistin resistance, which has attracted attention in recent years, was also investigated in this study.

2. Materials and methods

Salmonella spp. isolates from various foods, which cause food poisoning, were taken from mostly chickens and chicken products from food poisoning cases in Turkey. The biochemical properties of *Salmonella* spp. isolates were investigated in accordance with standard laboratory procedures (ISO 6579-1:2002). The isolates were identified as *Salmonella* spp. with VITEC II Compact. In this study, the ISO 6579-3 (Kaufmann-White scheme) method was used for serotyping of *Salmonella* spp. and for detection of antimicrobial resistance with MIC in line with EURL-AR [14]. MIC is used as the gold standard for determining

antimicrobial susceptibility of bacteria [15]. Therefore, the MIC test, which is the recommendation of EURL-AR, was preferred in this study.

According to this method, the cultures for contaminations were checked, then 3–4 colonies were picked and a suspension in 4 mL saline was prepared. The inoculum was prepared by transferring 50 µL of the suspension to 10 mL of Mueller Hinton Broth and the suspension was inoculated (50 µL per well) in the panels. The inoculum was approximately 5×10^5 CFU/mL. The panel with normal sealing was sealed. Purity control was performed by spreading a loop (1 µL) of the final suspension on a blood agar plate and the plate was incubated at 37 °C for 18–20 h.

The inoculums were separated into EUVSEC and EUVSEC2 panels using the Sensititre AIM Automated Inoculation Delivery System. The antimicrobial susceptibility was evaluated by determining the MIC by microdilution method using commercially available microplates, EUVSEC and EUVSEC2 panels (Sensititre, Trek Diagnostic Systems, Thermo Fisher Scientific, USA). All the isolates were phenotypically tested for their susceptibility to 14 antimicrobials from 9 different antimicrobial groups on EUVSEC: ampicillin (AMP), azithromycin (AZM), cefotaxime (FOT), ceftazidime (TAZ), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), gentamicin (GEN), meropenem (MER), nalidixic acid (NAL), sulfamethoxazole (SMX), tetracycline (TET), tigecycline (TGC), and trimetoprim (TMP). The results were monitored in a semiautomatic vision reader and evaluated according to EUCAST (European Committee on Antimicrobial Susceptibility Testing). The interpretive criteria were prepared according to the European Commission (EC) 2013/652/EU decision. Therefore, the following MIC values (EUCAST ECOFFs) were considered to indicate resistance to the first panel of antimicrobials: AMP > 8 mg/mL, AZM > 16 mg/mL, FOT > 0.5 mg/mL, TAZ > 2 mg/mL, CHL > 16 mg/mL, CIP ≥ 0.064 mg/mL, COL > 2 mg/mL, GEN > 2 mg/mL, MER > 0.125 mg/mL, NAL > 16 g/mL, SMX > 256 mg/mL, TET > 8 mg/mL, TGC >1 mg/mL, and TMP > 2 mg/mL. To phenotypically verify ESBL-/AmpC-/carbapenemase-producing *Salmonella* spp. isolates, they were tested with the second antimicrobial panel. EUCAST ECOFFs of the second panel are cefoxitin > 8 mg/L, cefepime (FEP) > 0.125 mg/L, MER > 0.125 mg/L, temocillin (TRM) > 32 mg/L, imipenem (IMI) > 1 mg/L, ertapenem (ERT) > 0.06 mg/L, CTX > 0.5 mg/L, and CAZ > 2 mg/L [14]. The isolates resistant to 3 or more antimicrobial classes were identified as multidrug-resistant [5].

Antimicrobials for *Salmonella* spp. were interpreted according to the criteria of the EC regulation 652/2013 for the production of ESBL, AmpC, or carbapenemase with cefotaxime, ceftazidime, or meropenem. Confirmatory tests are required for ESBL production in all isolates resistant to cefotaxime, ceftazidime, or meropenem, which must be done with the second antimicrobial panel. This panel includes the synergy test of cefoxitin, cefepime, and clavulanate along with FOT and CAZ for the detection of ESBL and AmpC production. Synergy is called for a 3-fold concentration decrease in MIC value (MIC FOT: FOT/Cl or TAZ : TAZ/Cl ratio ≥ 8) for the antimicrobial agent tested when the agent is tested alone in combination with clavulanic acid against MIC value. The presence of synergies suggests ESBL production. The detection of AmpC-type beta-lactamases is tested by looking at the susceptibility of the bacterium to FOX. Resistance to FOX indicates the presence of an Ampc-type beta-lactamase. In addition, the second panel looks at the resistance of IMI, MER, and ETP to phenotypically verify the hypothetical carbapenemase producer. The following MIC values were considered to indicate resistance: IMI: IMI > 1 µg/mL; MER: MER > 0.125 µg/mL, and ertapenem (ETP): ETP > 0.06 µg/mL [16].

Meropenem test should be performed to verify carbapenemase production. The classification of phenotypic results should be made according to the EFSA recommendations. The EUCAST cut-off values used to describe their resistance to FOT and CAZ are FOT > 0.5 and TAZ > 2. According to the panel 2 results, the values applied to identify bacterial phenotypes as ESBL, AmpC, or carbapenemase are FOT > 1 and TAZ > 1 [14].

Escherichia coli ATCC 25922 was used as the control strain as recommended. The results were interpreted according to the recommendations of the EUCAST and EURL-AR [5,14].

3. Results

This study examined resistance to 14 different antibiotics of 34 serotyped *Salmonella* spp. strains (2 *S.* Enteritidis, 5 *S.* Typhimurium, and 27 *S.* Infantis). Table 1 presents the values of the antibiotics and *Salmonella* spp. isolates used in the MIC test and Table 2 presents the antibiotic resistance properties of *Salmonella* spp. isolates detected by the MIC test. As shown in Table 3, the percentages of antibiotic resistance properties of *Salmonella* spp. isolates were detected by the MIC test.

Most of these isolates are resistant to ciprofloxacin, colistin, nalidixic acid, sulphonamides, tetracycline, tygecycline, and trimethoprim. Among these isolates, 3 were sensitive to all 14 antibiotics, 8 of them (23.5%) were determined to be resistant to a single antibiotic, and 20 of them (58.8%) were determined to be multidrug-resistant. Furthermore, it was found out that 19 out of 34 isolates (58.8%) were resistant to ciprofloxacin, 15 isolates (44.1%) to colistin, 19 isolates (58.8%) to nalidixic acid, 18 isolates

Table 1. The values of the antibiotics and *S.* Infantis isolates used in the MIC test.

Isolates	Antibiotics (µg/mL)													
	AMP >8	AZI >16	FOT >0.5	TAZ >2	CHL >16	CIP >0.064	COL >2	GEN >2	MER >0.125	NAL >16	SMX >256	TET >8	TGC >1	TMP >2
E. coli ATCC 25922	8	4	≤0.25	≤0.5	≤ 8	≤0.015	≤1	2	≤0.03	≤4	64	≤2	≤0.25	0.5
S. Infantis 2	2	4	≤0.25	≤0.5	≤ 8	0.5	2	≤0.5	≤0.03	>128	>1024	>64	1	>32
S. Infantis 12	32	≤2	>4	>8	≤ 8	0.06	>16	1	0.06	≤4	≤8	≤2	0.5	>32
S. Infantis 15	>64	8	≤0.25	≤0.5	≤ 8	0.5	2	≤0.5	≤0.03	>128	>1024	>64	1	>32
S. Infantis 29	4	8	≤0.25	≤0.5	≤ 8	1	2	≤0.5	≤0.03	128	64	64	2	32
S. Infantis 41	2	16	≤0.25	≤0.5	128	0.5	4	≤0.5	≤0.03	128	1024	64	1	32
S. Infantis 47	4	8	≤0.25	≤0.5	≤ 8	1	2	≤0.5	≤0.03	>128	>1024	>64	2	≤0.25
S. Infantis 51	4	8	≤0.25	≤0.5	≤ 8	1	2	≤0.5	≤0.03	128	1024	64	2	32
S. Infantis 52	>64	8	≤0.25	≤0.5	16	1	4	≤0.5	≤0.03	>128	>1024	>64	1	>32
S. Infantis 53	4	8	0.5	1	≤ 8	1	2	≤0.5	≤0.03	128	1024	64	2	≤0.25
S. Infantis 62N1	2	16	≤0.25	≤0.5	≤ 8	0.5	2	≤0.5	≤0.03	128	1024	64	1	32
S. Infantis 62N2	4	8	≤0.25	≤0.5	16	0.5	2	≤0.5	≤0.03	128	1024	64	2	32
S. Infantis 63	2	8	≤0.25	≤0.5	≤ 8	0.03	8	≤0.5	≤0.03	≤4	32	≤2	0.5	0.5
S. Infantis 64	2	8	≤0.25	≤0.5	≤ 8	≤0.015	2	≤0.5	≤0.03	≤4	32	≤2	0.5	≤0.25
S. Infantis 67N1	4	16	≤0.25	≤0.5	16	1	4	≤0.5	≤0.03	>128	>1024	>64	2	>32
S. Infantis 67N2	4	16	≤0.25	≤0.5	≤ 8	1	4	≤0.5	≤0.03	>128	1024	>64	2	>32
S. Infantis 67N3	4	8	≤0.25	≤0.5	>128	1	2	≤0.5	≤0.03	>128	>1024	>64	2	>32
S. Infantis 67N4	4	16	≤0.25	1	16	1	2	≤0.5	≤0.03	128	1024	64	2	32
S. Infantis 67N5	4	8	≤0.25	≤0.5	8	0.5	2	≤0.5	≤0.03	128	1024	64	1	32
S. Infantis 68	64	16	0.5	1	128	1	2	≤0.5	≤0.03	128	1024	64	8	32
S. Infantis 69	2	8	≤0.25	≤0.5	0.03	4	2	≤0.5	≤0.03	≤4	32	≤2	0.5	≤0.25
S. Infantis 70N1	2	8	≤0.25	≤0.5	≤ 8	0.03	8	≤0.5	≤0.03	≤4	32	≤2	0.5	0.5
S. Infantis 70N2	4	16	≤0.25	1	16	0.5	8	≤0.5	≤0.03	128	1024	64	2	4
S. Infantis 105	2	8	≤0.25	≤0.5	≤ 8	0.03	8	≤0.5	≤0.03	≤4	64	≤2	0.5	0.5
S. Infantis 109	≤1	4	≤0.25	≤0.5	≤ 8	0.25	2	≤0.5	≤0.03	128	1024	64	1	≤0.25
S. Infantis 114	2	8	≤0.25	≤0.5	≤ 8	0.03	8	≤0.5	≤0.03	≤4	32	≤2	0.5	0.5
S. Infantis 134	2	8	≤0.25	≤0.5	≤ 8	0.03	4	1	≤0.03	≤4	32	≤2	0.5	≤0.25
S. Infantis 142	4	8	0.5	1	≤ 8	0.03	4	1	≤0.03	≤4	64	4	0.5	0.5
S. Typhimurium 112	2	16	≤0.25	≤0.5	≤ 8	0.5	2	≤0.5	≤0.03	128	1024	64	2	≤0.25
S. Typhimurium 143	16	8	0.5	2	≤ 8	0.03	2	≤0.5	0.12	≤4	64	4	0.5	0.5
S. Typhimurium 144	4	8	0.5	1	≤ 8	0.03	4	≤0.5	0.06	≤4	64	4	0.5	0.5
S. Typhimurium 145	2	4	≤0.25	≤0.5	≤ 8	0.03	2	≤0.5	0.06	≤4	32	4	0.5	≤0.25
S. Typhimurium146	4	8	≤0.25	1	≤ 8	0.06	2	≤0.5	0.06	≤4	64	4	0.5	0.5
S. Enteritidis 16	2	4	≤0.25	≤0.5	≤ 8	0.03	8	≤0.5	≤0.03	8	32	≤2	0.5	0.5
S. Enteritidis 71	2	8	≤0.25	≤0.5	≤ 8	0.03	4	1	≤0.03	≤4	64	≤2	0.5	≤0.25

Ampicillin (AMP), Azithromycin (AZI), Cefotaxime (FOT), Ceftazidime (TAZ), Chloramphenicol (CHL), Ciprofloxacin (CIP), Colistin (COL), Gentamicin (GEN), Meropenem (MER), Nalidixic acid (NAL), Sulfonamides (SMX), Tetracycline (TET), Tigecycline (TGC), Trimetoprim (TMP).

Table 2. The antibiotic resistance properties of *S.* Infantis isolates detected by the MIC test.

Isolates	Antibiotics													
	AMP	AZI	FOT	TAZ	CHL	CIP	COL	GEN	MER	NAL	SMX	TET	TGC	TMP
E. coli ATCC 25922	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Infantis 2	S	S	S	S	S	R	S	S	S	R	R	R	S	R
S. Infantis 12	R	S	R	R	S	S	R	R	S	S	S	S	S	R
S. Infantis 15	R	S	S	S	S	R	S	S	S	R	R	R	S	R
S. Infantis 29	S	S	S	S	S	R	S	S	S	R	S	R	R	R
S. Infantis 41	S	S	S	S	R	R	R	S	S	R	R	R	S	R
S. Infantis 47	S	S	S	S	S	R	S	S	S	R	R	R	R	S
S. Infantis 51	S	S	S	S	S	R	S	S	S	R	R	R	R	R
S. Infantis 52	R	S	S	S	S	R	R	S	S	R	R	R	S	R
S. Infantis 53	S	S	S	S	S	R	S	S	S	R	R	R	R	S
S. Infantis 62N1	S	S	S	S	S	R	S	S	S	R	R	R	S	R
S. Infantis 62N2	S	S	S	S	S	R	S	S	S	R	R	R	R	R
S. Infantis 63	S	S	S	S	S	S	R	S	S	S	S	S	S	S
S. Infantis 64	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Infantis 67N1	S	S	S	S	S	R	R	S	S	R	R	R	R	R
S. Infantis 67N2	S	S	S	S	S	R	R	S	S	R	R	R	R	R
S. Infantis 67N3	S	S	S	S	R	R	S	S	S	R	R	R	R	R
S. Infantis 67N4	S	S	S	S	S	R	S	S	S	R	R	R	R	R
S. Infantis 67N5	S	S	S	S	S	R	S	S	S	R	R	R	S	R
S. Infantis 68	R	S	S	S	R	R	S	S	S	R	R	R	R	R
S. Infantis 69	S	S	S	S	S	R	S	**R**	S	S	S	S	S	S
S. Infantis 70N1	S	S	S	S	S	S	R	S	S	S	S	S	S	S
S. Infantis 70N2	S	S	S	S	S	R	R	S	S	R	R	R	R	R
S. Infantis 105	S	S	S	S	S	S	R	S	S	S	S	S	S	S
S. Infantis 109	S	S	S	S	S	R	S	S	S	R	R	R	S	S
S. Infantis 114	S	S	S	S	S	S	R	S	S	S	S	S	S	S
S. Infantis 134	S	S	S	S	S	S	R	S	S	S	S	S	S	S
S. Infantis 142	S	S	S	S	S	S	R	S	S	S	S	S	S	S
S. Typhimurium 112	S	S	S	S	S	R	S	S	S	R	R	R	R	S
S. Typhimurium 143	R	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Typhimurium 144	S	S	S	S	S	S	R	S	S	S	S	S	S	S
S. Typhimurium 145	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Typhimurium146	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Enteritidis 16	S	S	S	S	S	S	R	S	S	S	S	S	S	S
S. Enteritidis 71	S	S	S	S	S	S	R	S	S	S	S	S	S	S

Ampicillin (AMP), Azithromycin (AZI), Cefotaxime (FOT), Ceftazidime (TAZ), Chloramphenicol (CHL), Ciprofloxacin (CIP), Colistin (COL), Gentamicin (GEN), Meropenem (MER), Nalidixic acid (NAL), Sulfonamides (SMX), Tetracycline (TET), Tigecycline (TGC), Trimetoprim (TMP), Sensitive (S), Resistance (R).

Table 3. Percentages of antibiotic resistance properties of *Salmonella* spp. isolates detected by the MIC test.

Antibiotics	R/S	Resistant Strains (%)	Susceptible Strains (%)
AMP	5/29	14.7	85.3
AZI	0/34	0	100
FOT	1/33	2.9	97.1
TAZ	1/33	2.9	97.1
CHL	3/31	8.8	91.2
CIP	19/15	55.8	44.2
COL	15/19	44.1	55.9
GEN	1/33	2.9	97.1
MER	0/34	0	100
NAL	19/15	55.8	44.2
SMX	18/16	52.9	47.1
TET	19/15	55.8	44.2
TGC	12/22	35.2	64.8
TMP	16/18	47	53

Ampicillin (AMP), Azithromycin (AZI), Cefotaxime (FOT), Ceftazidime (TAZ), Chloramphenicol (CHL), Ciprofloxacin (CIP), Colistin (COL), Gentamicin (GEN), Meropenem (MER), Nalidixic acid (NAL), Sulfonamides (SMX), Tetracycline (TET), Tigecycline (TGC), Trimetoprim (TMP), Sensitive (S), Resistance (R).

(52.9%) to sulfonamides, 19 isolates (55.8%) to tetracycline, 12 isolates (35.2%) to tigecycline, and 16 strains (47%) to trimetoprim. Only 2 isolates were resistant to cefotaxime and ceftazidime; however, ESBL activity was not detected in the second stage of the analyses in which EUVSEC2 panels were used. All the isolates were determined as sensitive to cefepime. None of them were resistant to imipenem, meropenem, and ertapenem. Moreover, all the isolates for AmpC activities were determined to be sensitive to cefoxitin. Nalidixic acid, ciprofloxacin, and tetracycline resistance was the same. Colistin resistance was measured as 44.1%. *Salmonella* spp. isolates taken from food poisoning cases were found to have multidrug resistance to antibiotics at a high rate (58.8%). Only 4 *Salmonella* spp. isolates were found susceptible to all antibiotics in this study.

4. Discussion

Rapidly increasing antimicrobial resistance in *Salmonella* spp. is an important public health problem. It has been reported that antibiotics used in animals can lead to the development of resistant pathogens that infect humans throughout the food chain. Therefore, the use of antimicrobial agents in humans and animals needs to be performed with caution [17].

Researchers have reported that *Salmonella* spp. isolated from poultry carcasses poses a risk to human health. *Salmonella* spp. isolated from broiler farms has often been found susceptible to many of the antibiotics tested. Raseta et al. found no resistance to fluoroquinolones, high resistance to nalidixic acid, and low sensitivity to ciprofloxacin in their study [3]. In our study, the resistance to nalidixic acid, ciprofloxacin, and tetracycline was found to be 58.8%.

In a study in 2010, MIC values of 158 *Salmonella* spp. isolates were measured. Serovars were reported as *S.* Enteritidis (34%), *S.* Mbandaka (31%), and *S.* Infantis (12%). Antimicrobial resistance was determined to be 99.3% for gentamicin, 98.7% for cefotaxime, 97.5% for tetracycline, 95.5% for trimethoprim and ampicillin, 85.4% for ciprofloxacin, 95.4% for sulfamethoxazole, and 58% for nalidixic acid. In Croatia, 41.7% of isolates were susceptible to all antimicrobials, 43% of them were resistant to 1 antimicrobial, 12.7% were resistant to 2 antimicrobials, and 2.6% to 3 antimicrobials [18]. In our study, only nalidixic acid resistance was similar, but unlike in Croatia, the present study found less resistance to other antibiotics studied. Despite this, multidrug resistance was found to be higher in Turkey.

Nogrady et al. [19] concluded that *S.* Infantis clone, which has multiple resistance, is spread among humans through poultry meat. The strains in the study showed susceptibility to ciprofloxacin, while resistance values of 66.7%–100% were reported in Germany, Slovakia,

Bulgaria, and Austria. Compared to these countries, less resistance to ciprofloxacin has been detected in this study.

In 2013–2015 in Slovenia, the dominant *Salmonella* serovar was reported to be *S.* Infantis (92% and 100%, respectively). This study revealed a high resistance to ciprofloxacin (87.4%), sulfonamides (88.5%), tetracyclines (88.5%), nalidixic acid (87.4%), and streptomycin (72.4%). The results of this study are consistent with the extreme resistance to ciprofloxacin and nalidixic acid reported by many EU member states [5]. In addition, the present study showed multidrug resistance to ciprofloxacin, colistin, nalidixic acid, sulfonamides, tetracycline, and trimethoprim. Compared to EU countries, *S.* Infantis strains have lower resistance rates in Turkey.

In Serbia, resistance to *S.* Infantis was detected in poultry. According to this study, 8 isolates were resistant to nalidixic acid and tetracycline, 2 isolates were resistant only to nalidixic acid, and 8 isolates were found susceptible to all antibiotics used [20]. Resistance was detected to a greater number of varieties of antibiotics apart from the antibiotics reported in this study.

In another study, 84.6% of *S.* Infantis strains were susceptible to all antibiotics tested, 2 strains were tetracycline-resistant, and 2 strains were reported to have low β-lactam resistance [21]. ESBL activity was not detected in *S.* Infantis strains in this study.

Kudaka et al. [22] found that 99.2% of *S.* Infantis isolates and 0.8% of *S.* Enteritidis isolates from broilers in Japan had resistance to tetracycline, streptomycin, and trimetoprim, especially sulfonamide. *S.* Infantis resistance to nalidixic acid, sulfonamide, and tetracycline was 92.7%, 92.2%, and 88.3%, respectively. Additionally, *S.* Infantis resistance to quinolones was found to be high, similar to Germany and Hungary. Although ciprofloxacin rates are high in our country, they are well below the rates in these countries.

In a study conducted in Italy, ESBL-producing MDR *S.* Infantis was reported to spread from animals to humans. In this study, it was found that most isolates (92%) were resistant to at least one or more antimicrobials, and only 7 out of 87 isolates were susceptible to 14 antibiotics. Resistance was the highest to tetracycline in 88.5% of the isolates, to sulfonamides in 88.5%, and to nalidixic acid and ciprofloxacin in 87.4% of the isolates. Streptomycin resistance was reported to be remarkable (72.4%). In the same study, no resistance could be detected to third-generation cephalosporin, chloramphenicol, colistin, gentamicin, and trimethoprim and no ESBL was reported [5]. When the MDR results were compared with Italy, the rates of resistance to tetracycline, sulfonamide, and nalidixic acid were lower and resistance to colistin and trimethoprim was higher.

Another study carried out in Italy reported the existence of mcr-1-mediated colistin resistance in 4 multidrug resistant *S.* Infantis isolates, 2 of which were Extended Spectrum Beta-Lactamase (ESBL) producers, within the scope of the antimicrobial resistance monitoring program in broiler and broiler meats (2001–2017) [11]. Although ESBL activity was not detected in our study, colistin resistance was high (44.1%) in Turkey.

According to the MIC test, resistance rates were found to be 96.1% to nalidixic acid, 64.3% to enrofloxacin, 56.6% to ciprofloxacin, 34.1% to ofloxacin, and 30.2% to levofloxacin. In this study, quinolones/fluoroquinolones were used in the treatment of multidrug-resistant salmonellosis in "human and veterinary medicine" due to the high diversity in resistant clones and the detection of broad-spectrum antimicrobial activity. It is reported that increased antimicrobial resistance to quinolones/fluoroquinolones causes difficulties in controlling infections caused by *Salmonella* spp. [23]. In this study, resistance to nalidixic acid was found to be less, while ciprofloxacin resistance was at the same rates.

In a study conducted in Japan, fluoroquinolone-resistant *Salmonella spp.* strains were rarely detected in food-producing animals [13]. In another study in Brazil, 28.7% of the isolated *Salmonella* spp. strains were resistant to quinolones, 23.2% of them to ciprofloxacin, 12.4% to enrofloxacin and nalidixic acid, 1.5% to ciprofloxacin, and 30.2% only to nalidixic acid according to the MIC testing [24]. The present study also found a high resistance to ciprofloxacin (58.8%).

In a study conducted in Israel in 2014, cefotaxime, tetracycline, sulfonamides, trimethoprim, and ciprofloxacin-resistant *S.* Infantis clones that passed to humans through broiler-borne infections were isolated from chickens, chicken meat, and human samples. High rates of resistance to sulfonamide, trimethoprim, ciprofloxacin, and tetracycline were detected in *Salmonella* spp. strains, the majority of which were isolated from chickens and chicken products, as in this study. Most of the multidrug-resistant *S.* Infantis clones isolated from chicken and poultry products is reported to be a new ESBL-producing clone in Italy that spreads to broiler chickens [25]. ESBL activity in *Salmonella* spp. strains, the majority of which were isolated from chicken and chicken products, was not detected in this study.

Salmonella spp. was detected in 22% of meat samples taken from retail stores in Pennsylvania during 2006–-2007. Of these samples, 31% of the isolates were resistant to 3 antibiotics, 40% of the isolates were resistant to multiple antibiotics (4 antibiotics), 70% of them were resistant to at least one antibiotic, and 52% were resistant to ciprofloxacin [2]. On the other hand, 10 of them (29.4%) were determined as single and 20 of them (58.8%) were determined as multidrug resistant in this study.

Erdem et al. [26] found a high resistance to chloramphenicol, ampicillin, and sulfamethoxazole/

trimethoprim used in the treatment of *Salmonella* in Turkey. In this study, which was conducted between 2000 and 2010, compared to the previous years in Turkey, the resistance to ceftizoxime was seen in 12% of clinical isolates, and an increase of 12% was observed in the resistance to ceftazidime and ceftriaxone. According to this study, compared to 2017, the resistance of ciprofloxacin, nalidixic acid, sulfonamide, and trimethoprim continued to increase, but cefotaxime and ceftazidime resistance reported in other studies was not high in *Salmonella* spp. isolated from foods.

In their study, Acar et al. [17] detected multiple resistance to 2 or more antibiotics in 29% of isolates, and they reported multiple resistance of *S*. Enteritidis isolates in humans to be a major public health problem. In the study conducted in 9 different cities of Turkey, *Salmonella* strains isolated in Erzurum, Malatya, Denizli, and Alanya were all found to be susceptible to all the antibiotics studied. Among the cities, the highest level of resistance was found in strains from Kütahya and Ankara. In the present study conducted on *Salmonella* spp. strains, multidrug resistance increased significantly (58.8%).

In another study conducted in Turkey between the years 2012–2013, the antibiotic resistance of *Salmonella* serovar isolated from broiler chicken feces was investigated. Resistance rates were 42.2% for tetracycline, 42% for sulfonamide, 39.9% for trimethoprim, 36.9% for nalidixic acid, 32% for streptomycin, 31.5% for ampicillin, 15.3% for enrofloxacin, 11.5% for chloramphenicol, 10.2% for ciprofloxacin, 3.5% for gentamicin, 3.2% for cefotaxime, and 89.51% for multiple resistance. In conclusion, it was reported that the dominant serotype was *S*. Infantis and the antibiotic resistance rates were high in *Salmonella* spp. strains.

In conclusion, this is the first study carried out in Turkey that has evaluated ESBL, AmpC, and carbapenemase activity and colistin resistance of *Salmonella* spp., which was isolated from food poisoning cases, by using the MIC test as recommended by EURL-AR. As in other countries and compared to the previous years, it is seen that multidrug resistance has increased year by year in our country, but quite high resistance rates have not been determined yet as in other countries. ESBL, AmpC, and carbapenemase activities were determined in isolated *Salmonella* spp. strains. Although it is pleasing that resistance rates detected in isolates are not very high in our country, follow-up of these issues and monitoring the spread of antimicrobial resistance will help prevent future problems. In Turkey, antibiotics use should be avoided especially in chicken farms in order to prevent the increasing multidrug resistance to *Salmonella* spp. Studies should be performed routinely for the follow-up of antimicrobial resistant clones in Turkey.

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