Research article

ANTIBIOTIC RESISTANCE PROFILE OF *SALMONELLA ENTERICA* SUBSP. *ENTERICA* ISOLATED FROM DOG AND HUMAN SAMPLES IN TÜRKİYE: THE CASE OF KASTAMONU

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Salmonellosis is of great importance for humans and animals. Companion animals, particularly dogs, can be asymptomatic carriers of Salmonella, and thus have been ignored as a source of salmonellosis. They can also spread multidrug resistant Salmonella strains via dog feces, causing inconvenience in the treatment of human salmonellosis. The purpose of this study was to investigate the presence of Salmonella enterica subsp. enterica isolates from collected dog feces belonging to dogs residing at the Municipal Dog Shelter, Anatolian Shepherd Dog Farm, and from blood, stool, and joint fluid samples from humans with symptoms of gasroenteritis, abdominal, and joint pain. In addition to this, the antibiotic resistance profiles of Salmonella enterica subsp. enterica isolates were examined. A total of 45 human and 11 dog Salmonella enterica subsp. enterica isolates were obtained. The 11 Salmonella enterica subsp. enterica recovered from dogs were identified as S. Infantis, S. Enteritidis, and S. Typhimurium, which correspond to servars priotorized for human health. Almost all human isolates (42/45) and all dog Salmonella isolates (11/11) were found to be resistant to one or five, and one or four of the tested antibiotics, respectively, but not for CFZ, CAZ, CST in human and CFZ, CAZ, CST, ETP in dog Salmonella isolates. Common resistance profiles in dog and human origin Salmonella isolates were GEN/AMK, AMP/GEN/CIP/SXT, AMP/CIP, SXT. Exhibition of the common resistance profiles against antibiotics recommended in the treatment of human salmonellosis should not be ignored. Companion animals should be monitored for carrying Salmonella and spreading antibiotic resistant bacteria.

Keywords: antimicrobial resistance, dog, human, Salmonella enterica subsp. enterica

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INTRODUCTION

Salmonella spp. causes infections ranging from the subclinical carrier state to acute, severe septicemia in both humans and animals [1]. *Salmonella* spp. was reported as the second most common infectious agent, accounting for 60,050 confirmed human cases based on reported hospitalisations, fatalities from zoonoses in verified human cases, and cases of foodborne outbreaks in the EU by EFSA and CDC [2].

Although the majority of Salmonella cases in humans was attributed to consumed poultry meat and products, companion animals can also be a source of Salmonella spp. for humans [3-6]. Taking into account of all the information gathered on the presence of Salmonella in various categories of animal species in the EU, 70,326 samples taken from animals of different species (excluding poultry) were examined for the presence of Salmonella. 4% and 4.5% of all samples tested were positive for Salmonella (N = 2,843) in EU Member States (MS) and non-MS, respectively [2,7]. According to EFSA and CDC data, the percentage of dogs positive for Salmonella reported by MS and non-MS countries were 2.7% (53/1.995) and 4.3% (46/1.082), respectively [7]. Dogs are the most preferred pets by humans among companion animals in the world [8]. Dogs known as asymptomatic carriers of Salmonella spp. can intermittently shed bacteria for more than six weeks. In addition to this, Salmonella spp. exists in the intestine and mesenteric lymph nodes of dogs without clinical signs [9]. In different areas of the world including United States [10], United Kingdom [11], Thailand [12], Taiwan [13], Turkey [14,15], Triniad [16], Ethiopia [1], the presence of Salmonella spp. in dogs was identified.

The top five *Salmonella* serovars causing human infections were generally distributed as follows: *Salmonella enterica* subspecies *enterica serovar* Enteritidis (*S*. Enteritidis) (54.6%), *S*. Typhimurium (11.4%), monophasic *S*. Typhimurium (8.8%), *S*. Infantis (2.0%) and *S*. Derby (0.93%) [2]. In a study in the United States, *S*. Typhimurium was found to share the third place with *S*. Infantis, following *S*. Enteritidis, which shared the second place with *S*. Javana, whereas *S*. Derby was the serovar with the lowest prevalence. The serovars frequently isolated from humans have also been found in dog feces [10].

The increasing prevalence of antimicrobial-resistant bacteria (AMR) and the advent of zoonotic bacterial diseases increase global concerns. Antimicrobial-resistant bacteria are a major source of concern due to their detrimental effects on domestic animals and humans. As a result, it is necessary to monitor these pathogens to protect human biosecurity [17]. The issue is that as bacteria adapt to environmental conditions quickly, they build drug resistance in a short time, and resistance genes can be horizontally shared throughout bacterial species, allowing bacteria to develop resistance quickly [18]. The primary cause of antibiotic resistance is the overdose and irresponsible usage of antibiotics as growth promoters in animal feeds, during treatment, and as a preventative measure [19]. As a result, the animals become carriers of bacteria that are resistant to antibiotics [17]. The major way that antibiotic-resistant bacteria infect humans is through fecal contamination as these bacteria are located in the gut. Resistant

zoonotic bacteria may spread to humans through animal-to-human contact and the environment in which they are raised. Given that the same multi-resistant *Salmonella* strains are circulating between human and dog populations and spread through close contact, dogs are considered a significant public health threat [1,15].

The aim of this study was to investigate the presence of *Salmonella* serovars in Municipal Dog Shelter (MDS) and the Anatolian Shepherd Dog Farm (ASDF), and distribution of human and dog origin *Salmonella* antibiotic resistant profiles against the antibiotics used in the treatment of human salmonellosis.

MATERIAL AND METHODS

Dog Fecal Samples

All fecal samples were collected from apparently healthy dogs on two different locations in Kastamonu-Türkiye. At the Municipal Dog Shelter 65 fecal samples were collected from 27 female and 38 male dogs. Other samples were collected from the Anatolian Shepherd Dog Farm. Seven out of 27 female dog samples belonged to dogs younger than one year of age. Twenty out of 27 female dog samples belonged to dogs at least one year of age. A total of 38 fecal samples were collected from male dogs: nine from those under one year old and 29 from those at least one year old. Thirty-five samples belonged to 19 female (4 from <1 year old, 15 from \geq 1 year old female dogs) and 16 male dogs (5 from <1 year old, 11 from \geq 1 year old male dogs) were collected from the Anatolian Shepherd Dog Farm (Table 1).

S	Number of	female dogs	Number of male dogs						
Sampling area	<1 year old	≥1 year old	<1 year old	≥1 year old					
MDS	7	20	9	29					
ASDF	4	15	5	11					
TOTAL	11	35	14	40					

Table 1. Fecal samples collected from MDS and ASDF

MDS: Municipal Dog Shelter; ASDF: Anatolian Shepherd Dog Farm

Description of housing and feeding at the sampling sites

Four or five dogs resided in the same kennel at MDS regardless of their age, gender or physical condition. The dogs were fed with household leftovers (vegetables, meals, cooked chicken, and cooked beef meat etc...). The hygienic conditions were not satisfactory. The housing conditions at ASDF were found to be more scrupulous, with each dog residing in a different kennel. The environmental conditions of ASDF were hygenic, with the feces being removed from the kennels every morning. The dogs were fed with raw chicken meat. The major difference between the two places was the environmental and housing conditions.

Culture of human fecal samples and antibiotic susceptibility test

For the culture of human samples and antibiotic susceptibility test, 45 samples including fecal samples (n=29), blood (n=12), and joint fluid (n=4) were collected from patients who have complaints of gastroenteritis, abdominal and joint pain at Izmir Katip Celebi University, Ataturk Training and Research Hospital. All the samples were inoculated on Eosin Methylene Blue Agar (Becton Dickinson, USA), and 5% Sheep Blood Agar (Becton Dickinson, USA). Agar plates were incubated at 37°C for 18-24 hours. Only one instance of growth was considered when the same microorganism appeared in multiple samples from the same patient. Strains found to be bacteria in Gram staining were evaluated by colony morphology and biochemical tests (oxidase, coagulase, and catalase test, three sugar iron test, urea hydrolysis test, indole, methyl - red, citrate test). Identification (MALDITOF MS, BD, USA) and antibiotic susceptibility test of isolated bacteria (Phoenix, BD, USA) were performed using an automated system. The results of the antibiotic susceptibility test were evaluated according to the European Committee on Antimicrobial Susceptibility Testing Standards (EUCAST) [20]. In our country, antimicrobial susceptibility testing (AST) application and reporting have transitioned to EUCAST [20]. However, the limited antibiogram lists that were found at the Clinical and Laboratory Standards Institute (CLSI) documents are not available in EUCAST [21]. Therefore, the TMC-ADTS (Turkish Medical Consortium – Antimicrobial Drug Testing Study) group has prepared limited notification tables that can be used in conjunction with EUCAST standards, taking into account the conditions in our country and the recommendations in the CLSI documents. The first of these tables was published in 2016 and updated in 2022 [22,23]. In the limited notification list, antibiotics from groups A, B, and C that are commonly used in both companion animals and humans for the treatment of nonurinary infections caused by bacteria from Enterobacteriaceae family have been selected in this study. Group A (ampicillin, cephazoline, gentamicin) includes drugs that require priority testing and reporting. Group B (amoxicillin/clavulanic acid, ceftazidime, ciprofloxacin, trimethoprim-sulfamethoxazole) includes drugs that should be tested with limited reporting. Group C (colistin) drugs are subject to special conditions [22,23].

Culture of dog fecal samples and antibiotic susceptibility test

Dog fecal samples randomly collected in the morning were examined according to ISO 6579:2002/Amd-1:2007 (Annex D) [24]. Each 25 g feces was added to 225 mL of buffered peptone water (BPW, Oxoid CM509), and incubated at 37 (±1) °C for 16-18 hours for pre-enrichment. After incubation, 1 mL and 0.1 mL of each preenrichment culture were inoculated into Mueller Kaufmann Tetrathionate Novobiocin Broth (Oxoid; CM1048) and Modified Semi Solid Rappaport Vassiliadis Medium (HiMedia, M1428), respectively. Mueller Kaufmann Tetrathionate Novobiocin Broth (Oxoid; CM1048) and Modified Semi Solid Rappaport Vassiliadis Medium (HiMedia, M1428) enrichment cultures were incubated at 37 °C and 41.5 °C, respectively. After enrichment, a loopful of inoculum was streaked on each Brilliant Green Agar (Oxoid, CM0263) and XLD agar (Oxoid, CM0469). Pure cultures were prepared from the suspected Salmonella colonies in Brain Hearth Infusion broth (Oxoid; CM1135). In order to identify the pure cultures of suspected colonies, the biochemical tests were used: triple sugar iron (Oxoid, CM0277), urea hydrolysis (Oxoid, CM0053B), H₂S, indole production, ONPG (β-galactosidase; Oxoid, DD0013), lysine decarboxylase (Oxoid, CM038) and Voges Proskauer (Oxoid, CM0043). Polyvalent and monovalent specific somatic and flagellar antisera (Statens Serum Institute, Denmark) were used to confirm and serotype all identified Salmonella spp. isolates according to Kauffmann-White Scheme [25]. All Salmonella enterica subsp. enterica isolates of dogs were tested for antimicrobial susceptibility by using the Kirby Bauer disk diffusion method according to the guidelines of the CLSI [26] and EUCAST [20]. In the limited notification list, antibiotics from groups A, B, and C that are commonly used in both companion animals and humans for non-urinary samples in Enterobacteriaceae as described in the previous section were selected in this study [23]. All of the isolates were screened for resistance by using antibiotic discs (Oxoid). The antibiotics were ampicillin (AMP 10 µg), cephazolin (CFZ 30 µg), gentamicin (GEN 10 µg), amoxillin/cluvalanic acid (AMC 20/10 µg), ceftazidime (CAZ 30 µg), ciprofloxacin (CIP 5 µg), trimethoprimsulfamethoxazole (SXT 25 µg), amikacin (AMK 30 µg), colisin (CST 10 µg), ertapenem (ETP 10µg). Escherichia coli ATCC 25922 and Salmonella Typhimurium ATCC 14028 were used as the negative and positive control, respectively.

Statistical analysis

The animals were divided into two age groups: animals aged equal and up to 1 years, and less than 1 years. Association between the age and *Salmonella* carriage status was evaluated by Chi Square Test. For statistic analysis, Statistical package IBM SPSS23 was used.

RESULTS

A total of 100 dog fecal samples, comprising 65 from the Municipal Dog Shelter (27 from female and 38 from male dogs) and 35 from the Anatolian Shepherd Dog Farm (19 from female and 16 from male dogs), were examined for the presence of *Salmonella* spp. *Salmonella* spp. were found in eleven of the tested samples (11%). There was not a significant relationship between the animal age and *Salmonella* carriage status (p> 0.05). Among 11 *Salmonella* isolates, three distinct serovars – *S*. Enteritidis, *S*. Typhimurium, and *S*. Infantis were determined. Three *S*. Enteritidis were isolated from the feces of dogs younger than one year of age (two from male dogs, one from a female dog) from the same kennel at MDS (Table 2). Two *S*. Typhimurium (diphasic) were obtained from ≥ 1 year old male dogs resided at the same kennel of MDS. Four of six *S*. Infantis were isolated from each two ≥ 1 year old male (2/4) and female (2/4) dogs resided at

the same kennel of MDS, the other two *S*. Infantis were found from ≥ 1 year old male dogs resided at the individual kennels of ASDF (Table 2).

		М	DS			AS	SDF	
	<1 yea	ar old	≥1 yea	ar old	<1 yea	ar old	≥1 yea	ar old
	Female	Male	Female	Male	Female	Male	Female	Male
S. Enteritidis	1	2	-	-	-	-	-	-
S. Typhimurium	-	-	-	2	-	-	-	-
S. Infantis	-	-	2	2	-	-	-	2

Table 2. Salmonella serovars isolated from fecal samples of dogs

MDS: Municipal Dog Shelter; ASDF: Anatolian Shepherd Dog Farm; -: No growth

All human isolates were determined to be *Salmonella* spp. The identification scores of 13 of 45 isolates were determined to be above 2.30 and the remaining were detected between 2 and 2.30 with the MALDI TOF system. Serotyping was not performed on human *Salmonella* isolates. Almost all human *Salmonella* isolates (42/45) were found to be resistant to one or four of the antibiotics tested except for three isolates (HS14, HS15, HS16). The highest to lowest resistant rates were observed against GEN (24.44%), AMK (20.00%), CIP (17.77%), AMC (11.11%), SXT (6.66%), AMC (1.45%), and ETP (1.45%), respectively, while all human isolates were found to be sensitive to CFZ, CAZ, CST (Table 3). GEN/AMK/ETP, GEN/CIP/AMK, AMP/GEN/AMC/AMK, AMP/GEN/AMK, CIP/SXT, AMP/GEN/CIP, and CIP resistance patterns were determined with the rate of 2.38% (1/42), 14.28% (6/42), 4.76% (2/42), 4.76% (2/42), 2.38% (1/42), and 2.38% (1/42) in solely human resistant *Salmonella* isolates, but not for dog *Salmonella* isolates (Table 3).

All *Salmonella* isolates originating from dogs were found to be resistant to between one and four of the antibiotics tested except for CFZ, CAZ, CST, ETP (Tabel 4). It was determined that both GEN and SXT shared the second place in terms of resistancy with the rate of 54.54%, following AMP with the rate of 63.63% in dog *Salmonella* isolates (Table 4). The resistance rates of other AMK and AMC were determined to be 18.18% and 9.09%, respectively (Table 4).

The combined resistance patterns observed in both dog and human *Salmonella* isolates were GEN/AMK, AMP/GEN/CIP/SXT, AMP/CIP, and SXT (Table 5). The same combined resistance pattern GEN/AMK recovered from dog feces (one *S*. Infantis), and 24 human origin *Salmonella* isolates with the rates of 9.09% and 54.76%, respectively. Four dog origin *Salmonella* isolates (3 *S*. Enteritidis, 1 *S*. Infantis) and two human origin *Salmonella* spp. were found to be resistant to AMP/GEN/CIP/SXT with the rates of 36.36% and 4.76%, respectively. AMP/CIP combined resistance pattern was observed in two dog origin *Salmonella* isolates (one *S*. Typhimurium and one *S*. Infantis) (27.27%) and one human origin *Salmonella* spp. were found to be resistant to single antibiotic, SXT. In this study, GEN/AMK, AMP/GEN/CIP/SXT, AMP/CIP, SXT resistance profiles were observed in both human and dog origin *Salmonella* isolates (Table 5).

_	TP	S	S	К	S	S	S	S	S	S	S	S	S	S	S	S	S	/45 22%)
Group	Ē																	1, (2.:
C	CST	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0/45 (0.00)
	AMK	К	S	Я	S	К	R	R	R	R	R	S	S	S	S	R	S	9/45 (20.00%)
	SXT	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	R	3/45 (6.66%)
B Group	CIP	S	S	S	R	К	S	К	S	S	R	R	R	R	R	S	S	8/45 (17.77)
	CAZ	s	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0/45 (0.00)
	AMC	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	1/45 (2.22%)
	GEN	К	S	R	S	R	R	R	R	R	R	R	S	S	R	R	S	11/45 (24.44%)
A Group	CFZ	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0/45 (0.00)
	AMP	S	S	S	S	S	S	S	R	R	S	R	R	S	R	S	S	5/45 (11.11%)
	Sampling	Feces	Feces	Feces	Feces	Feces	Blood	Blood	Joint fluid	Joint fluid	Blood	Blood	Blood	Blood	Blood	Blood	Blood	
	Code of Salmonella spp.	HS1-HS13	HS14-HS16	HS17	HS18	HS19	HS20 – HS28	HS29	HS30,HS31	HS32,HS33	HS34-HS37	HS38,HS39	HS40	HS41,HS42	HS43	HS44	HS45	General total (%)

Table 3. Antibiotic resistance profiles of the Salmonella spp. strains (HS) isolated from human samples

AMP: Ampicillin; CFZ: Cephazolin; GEN: Gentamicin; AMC: Amoxillin/cluvalanic acid; CAZ: Ceftazidime; CIP: Ciprofloxacin; SXT: Trimethoprim-sulfamethoxazole; AMK: Amikacin; CST: Colisin; ETP: Ertapenem; HS: Salmonella of human origin

Salmonella			A Group				B Group			C G	dno
serovars	Sampling	AMP	CFZ	GEN	AMC	CAZ	CIP	SXT	AMK	CST	ETP
S. Enteritidis	MSD/feces	К	S	R	S	S	R	R	S	s	S
S. Enteritidis	MSD/feces	R	S	R	S	S	R	R	S	S	S
S. Enteritidis	MSD/feces	К	S	R	R	S	R	S	R	S	S
S. Typhimurium	MSD/feces	R	S	S	S	S	R	S	S	S	S
S. Typhimurium	MSD/feces	R	S	R	S	S	S	R	S	S	S
S. Infantis	MSD/feces	R	S	S	S	S	R	S	S	S	S
S. Infantis	MSD/feces	К	S	R	S	S	S	R	S	S	S
S. Infantis	MSD/feces	S	S	R	S	S	S	S	R	S	S
S. Infantis	MSD/feces	R	S	R	S	S	S	R	S	S	S
S. Infantis	ASDF/feces	S	S	S	S	S	S	R	S	S	S
S. Infantis	ASDF/feces	S	S	S	S	S	S	R	S	S	S
General Total (%)		7/11 (63.63)	0/11 (0.00)	6/11 (54.54)	1/11 (9.09)	0/11 (0.00)	5/11 (45.45)	6/11 (54.54)	2/11 (18.18)	0/11 (0.00)	0/11 (0.00)
MDS: Municipal I AMC: Amoxillin/(CST: Colisin; ETT	Dog Shelter; AS cluvalanic acid; ?: Ertapenem	DF: Anato CAZ: Ceft	ılian Sheph azidime; C	terd Dog Fa IP: Ciprofi	arm; AMP: oxacin; SX	: Ampicillir T: Trimetk	ı; CFZ: Ce ıoprim-sulf	phazolin; C amethoxaz	JEN: Gent ole; AMK: .	amicin; Amikacin;	

Table 4. Antibiotic resistance profiles of the Salmonella serovars isolated from dogs

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itance rn	Code of HS	Number of HS (%)	Name of DS	Number of DS (%)	General Total (%)
MK	HS1-HS13, HS44, HS20-HS28	23/42 (54.76)	1 S. Infantis	1/11 (9.09)	24/53 (54.28)
MK/ETP	HS17	1/42 (2.38)	ı	0/11 (0.00)	0/53 (0.00)
	HS18	1/42 (2.38)	ı	0/11 (0.00)	0/53~(0.00)
IP/AMK	HS34-HS37, HS19, HS29	6/42 (14.28)	ı	0/11 (0.00)	0/53~(0.00)
EN/AMC/	HS30, HS31	2/42 (4.76)	ı	0/11 (0.00)	0/53~(0.00)
EN/AMK	HS32, HS33	2/42 (4.76)	I	0/11 (0.00)	0/53 (0.00)
EN/CIP/	HS38, HS39	2/42 (4.76)	3 S. Enteritidis 1 S. Kentucky	4/11 (36.36)	6/53 (11.32)
IP	HS40	1/42 (2.38)	1 S. Typhimurium 1 S. Infantis	3/11 (27.27)	0/53~(0.00)
Γ	HS41, HS42	2/42 (4.76)	i.	0/11 (0.00)	0/53~(0.00)
EN/CIP	HS43	1/42 (2.38)	I	0/11 (0.00)	0/53~(0.00)
	HS45	1/42 (2.38)	2 S. Infantis	2/11 (18.18)	3/53 (5.66)
EN/SXT	I	I	1 S. Typhimurium 1 S. Infantis	2/11 (18.18)	2/53 (3.77)

Table 5. Resistance patterns of human and dog Salmonella spp. isolates

AMP: Ampicillin; CFZ: Cephazolin; GEN: Gentamicin; AMC: Amoxillin/cluvalanic acid; CAZ: Ceftazidime; CIP: Ciprofloxacin; SXT: Trimethoprim-sulfamethoxazole; AMK: Amikacin; CST: Colisin; ETP: Ertapenem; HS: Salmonella of human origin; DS, Salmonella serovars of dog

DISCUSSION

In this study, the subclinical shedding of *Salmonella* spp. in dogs with the rate of 11% was found to be higher than EU Member States (2.7%) [2], USA (2.5%) [10], United Kingdom (0.2%) [27], Ethiopia (5.5%) [1], China (9.47%) [28], Mexico (6.27%) [29], India (5.6%) [30]; in line with Iran and Addis Abbaba, Ethipia with rates of 10.5% [31] and 11.7% [32], China (3.6%) [33] respectively; lower than Nigeria (43.7%) [34], USA (20.8%) [35], Canada (23%) [36]. The prevalence of *Salmonella* spp. ranging between 0.2-43.7% in the previous studies was thought to rely on factors such as pet sanitary practices, feeding habits, public health awareness of *Salmonella* risk factors, differences on socioeconomic status of owners, environment of studies conducted, the season of the studies, and diagnostic methods [1,37].

The presence of *Salmonella* spp. in animals was associated with their age, as younger dogs showed a higher prevalence of *Salmonella* than older dogs in a study in Hangzhou, China [33], which may be explained by their immature gut microbiota or immune system [38,39]. However, it was determined that there was no significant relationship between the animal ages and *Salmonella* carriage status (p > 0.05).

Three distinct serovars -S. Enteritidis, S. Typhimurium, and S. Infantis – coinciding with the serovars prioritized for human health, were identified from fecal cultures of dog feces in this study. The transmission of zoonotic pathogens from pets to their owners is a distinct threat to human health [40]. A high degree of serotype diversity was observed between the studies such as reports of S. Typhimurium, S. Newport, S. Javiana from USA [10], S. Kentucky, S. Indiana, S. Typhimurium, S. Toucra, S. Sandiego, S. Newport, S. Saintpaul from Xuzhou, China [28], S. Dublin and S. Typhimurium from Hangzhou, China [33], S. enterica subspecies arizonae from Midlands Region of the United Kingdom [27], S. Enteritidis from USA [41] and Turkey [15], S. Corvallis from Turkey [14]. Two S. Typhimurium (diphasic) were observed in ≥ 1 year old male dogs resided at the same kennel of MDS. The same serovars in addition to S. Senftenberg were reported to be detected in dog feces in Tahran [42], corroborating our results. Two S. Typhimurium, three S. Enteritidis, and six S. Infantis samples observed in dog feces in this study are of great importance as they coincided with three of the five serovars that are prioritized for public health in terms of food safety monitoring in Europe [43,44].

In a study conducted in Turkey, S. Enteritidis (n=23) and S. Infantis (n=14) were the most frequently isolated serovars in humans [45]. Dogs at MDS and ASDF were fed with household leftovers and/or raw chicken meat, respectively. In this study, the shedding of serovars through dog feces, which poses a threat to public health, was attributed to dogs being fed *Salmonella*-contaminated chicken meat. Similar to the results of this study, a study in UK found that 4% of dogs (8/190) harboured *Salmonella* species in their feces, all of which were fed with raw food [46]. According to European Union One Health 2021 Zoonoses Report food-animal sources, a total of 20,020 serotyped isolates were reported from which S. Infantis accounted for 33.9%, S. Enteritidis for 8.2%, S. Typhimurium for 3.8%. Salmonella serovars were isolated from various food-animal sources. S. Typhimurium is found in several sources, but is more prevalent in broilers and pigs. S. Enteritidis and S. Infantis are mostly linked to broiler sources (chickens) [2,7]. Moreover, in a retrospective study conducted between 2013 and 2017, nearly 31 S. Infantis food poisoning cases in Turkey were originated from chicken meat [47]. S. Infantis contamination in chicken meat was declared to be 86.18% in Turkey [48]. Two S. Infantis in dog feces were thought to be the result of feeding dogs with raw chicken meat in the Anatolian Shepherd Dog Farm similiar to the results of Yukawa et al. [49], who reported S. Infantis, S. Schwanzengrund and untypeable Salmonella of seven raw-chicken meat based dog food in Japan. However, in our study, chicken meat was not examined for the presence of Salmonella, as the isolation of S. Infantis from dog feces was attributed to the raw chicken meat feeding practice. S. Enteritidis and S. Tyhimurium load of dog feces at the Municipal Dog Shelter might be due to carrying a clone of the same serovars because they were housed in the same kennel in groups of four or five. Moreover, the dogs at the Municipal Dog Shelter which had S. Enteritidis and S. Typhimurium load in their feces were formerly stray dogs, indicating that one of them could have been infected with Salmonella serovars before entering into the kennel and disseminate bacteria via feces to the other dogs sharing the same kennel [50].

In a study conducted in China, the resistance rates of Salmonella strains isolated from dogs and cats were found to be 80%, 64%, and 36% against AMP, GEN, and SXT, respectively when compared with the antibiotics that exhibit first three high resistances in this study [28]. Usmael et al. [1] declared high AMP resistance rate (41.7%) in dog Salmonella isolates similar to this study, but not for SXT (4.02%) and GEN (0.00%). Bataller et al. [37] solely reported AMP resistance in one of three dog Salmonella isolates, and the isolates were declared to be susceptible of the tested antibiotics: CTX, CAZ, GM, ND. CIP, AZM, TGC, SXT, CT, C in Southern Spain which was not compatible with the results in this study since the dog Salmonella isolates were resistant to at least one antibiotic. AMP, GEN, and SXT resistance was associated with the common usage of antibiotics for a long period of time in order to cure bacterial infections such as Salmonella [18]. Hence, observed resistance against them was interpreted as inevitable. According to a meta-analysis study conducted in Africa, the lack of CIP resistance and 33.30% AMC resistance rate in dog feces were shown to be incompatible with the results of this study with 45.45% CIP and 9.09% AMC resistance rates, respectively [51]. AMP/GEN/SXT resistance pattern was determined in one S. Typhimurium and one S. Infantis, which is found in dog feces, but not in human isolates (Table 4).

Salmonella infections in humans were associated with food-producing animals such as swine, poultry, and cattle. The infections by *Salmonella* serovars in companion animals are relatively neglected. A number of studies on prevalence and resistance patterns in dogs, their owners, as well as veterinary clinics and their employees had been reported, comprising of Multi Drug Resistance (MDR) *Salmonella* serovars such as MDR *S*. Typhimurium phage type DT104 in different hosts including humans and animals [52].

Dogs carry zoonotic bacterial pathogens and shed multidrug resistant enteric bacteria in their feces which may pose public health risks [53,54].

The exhibition of combined resistance profiles was as expected, as aminoglycosides, beta-lactams, fluoroquinolones, sulfanomides which are commonly used to treat various bacterial infections in both humans and dogs [7]. When compared to a previous study, the resistance profile of AMP/GEN/CIP/SXT shared common resistance pattern withby AMP-CHL-CIP-NA-SXT-TET, along with additional antimicrobials [55].

CONCLUSION

Companion animals, particularly dogs, should be examined for the presence of *Salmonella* as the second most commonly agent that caused infections in humans. *S*. Typhimurium, *S*. Enteritidis, and *S*. Infantis load of dog feces in the study are of great importance as they are correspond to three of the five serovars that are prioritized for public health. GEN/AMK, AMP/GEN/CIP/SXT, AMP/CIP common resistance profiles shared in both human and dog isolates in the study should be considered as a threat in terms of curing human Salmonellosis cases. Dogs that are asymptomatic carriers of *Salmonella* spp. should not be disregarded since the close relationship between dogs and humans. We anticipate that, companion animals in Turkey will also need to be examined for MDR *Salmonella*, in addition to farm animals farmed for food.

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Authors' contributions

EG was the student project's supervisor, planning and writing the whole manuscript and identifying antibiograms of dog samples. PMK carried out serotyping, statistical analysis, and manuscript editing. AE collected dog fecal samples and planned and wrote the project with supervisor EG. IK carried out collecting the samples and isolation. FBB carried out the isolation, identification, and writing of materials and methods sections for human samples. TB evaluated the antibiogram results of human samples.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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REFERENCES

- 1. Usmael B, Abraha B, Alemu S, Mummed B, Hiko AA, Abdurehman A: Isolation, antimicrobial susceptibility patterns, and risk factors assessment of non-typhoidal *Salmonella* from apparently healthy and diarrheic dogs. BMC Vet Res 2022, 18(1): 37.
- 2. European Food Safety Authority, European Centre for Disease Prevention and Control: The European Union One Health 2021 Zoonoses Report. EFSA J 2022, 20(12): e07666.
- Anderson TC, Marsden-Haug N, Morris JF, Culpepper W, Bessette N, Adams JK, Bidol S, Meyer S, Schmitz J, Erdman MM, Gomez TM, Barton Behravesh C: Multistate outbreak of human *Salmonella* Typhimurium infections linked to pet hedgehogs – United States, 2011-2013. Zoonoses Public Health 2017, 64: 290-298.
- 4. Bruce HL, Barrow PA, Rycroft AN: Zoonotic potential of *Salmonella* enterica carried by pet tortoises. Vet Rec 2018, 182(5): 141.
- Damborg P, Broens EM, Chomel BB, Guenther S, Pasmans F, Wagenaar JA, Weese JS, Wieler LH, Windahl U, Vanrompay D, Guardabassi L: Bacterial zoonoses transmitted by household pets: state-of-the-art and future perspectives for targeted research and policy actions. J Comp Pathol 2016, 155: (1 Suppl 1): S27-40.
- 6. Finlay F, Furnell C, Ridley P: *Salmonella* in pets: the risk to children. Community Pract 2015, 88 (7): 27-28.
- European Food Safety Authority, European Centre for Disease Prevention and Control: The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2019/2020. EFSA J 2022, 20(3): e07209.
- 8. Westgarth C, Pinchbeck GL, Bradshaw JW, Dawson S, Gaskell RM, Christley RM: Factors associated with dog ownership and contact with dogs in a UK community. BMC Vet Res 2007, 3: 5.
- 9. Leonard F: *Salmonella* infection and carriage: the importance of dogs and their owners. Vet Rec 2014, 174(4): 92-93.
- 10. Reimschuessel R, Grabenstein M, Guag J, Nemser SM, Song K, Qiu J, Clothier KA, Byrne BA, Marks SL, Cadmus K, Pabilonia K, Sanchez S, Rajeev S, Ensley S, Frana TS, Jergens AE, Chappell KH, Thakur S, Byrum B, Cui J, Zhang Y, Erdman MM, Rankin SC, Daly R, Das S, Ruesch L, Lawhon SD, Zhang S, Baszler T, Diaz-Campos D, Hartmann F, Okwumabua O: Multilaboratory survey to evaluate *Salmonella* prevalence in diarrheic and

nondiarrheic dogs and cats in the United States between 2012 and 2014. J Clin Microbiol 2017, 55(5): 1350-1368.

- 11. Philbey AW, Mather HA, Gibbons JF, Thompson H, Taylor DJ, Coia JE: Serovars, bacteriophage types and antimicrobial sensitivities associated with salmonellosis in dogs in the UK (1954-2012). Vet Rec 2014, 174(4):94.
- 12. Srisanga S, Angkititrakul S, Sringam P, Le Ho PT, At TV, Chuanchuen R: Phenotypic and genotypic antimicrobial resistance and virulence genes of *Salmonella* enterica isolated from pet dogs and cats. J Vet Sci 2017, 18(3):273-281.
- 13. Tsai HJ, Huang HC, Lin CM, Lien YY, Chou CH: Salmonellae and campylobacters in household and stray dogs in northern Taiwan. Vet Res Commun 2007, 31(8): 931-939.
- Kocabiyik AL, Cetin C, Dedicova D: Detection of *Salmonella* spp. in stray dogs in Bursa Province, Turkey: first isolation of *Salmonella* Corvallis from dogs. Vet Med B Infect Dis Vet Public Health 2006, 53(4):194-196.
- 15. Bagcigil AF, Ikiz S, Dokuzeylul B, Basaran B, Or E, Ozgur NY: Fecal shedding of *Salmonella* spp. in dogs. J Vet Med Sci 2007, 69(7): 775-777.
- 16. Seepersadsingh N, Adesiyun AA, Seebaransingh R: Prevalence and antimicrobial resistance of *Salmonella* spp. in non-diarrhoeic dogs in Trinidad. J Vet Med B Infect Dis Vet Public Health 2004, 51(7): 337-342.
- 17. Mthembu TP, Zishiri OT, El Zowalaty ME: Molecular detection of multidrug-resistant *Salmonella* isolated from livestock production systems in South Africa. Infect Drug Resist 2019, 12: 3537-3548.
- Deris JB, Kim M, Zhang Z, Okano H, Hermsen R, Groisman A, Hwa T: The innate growth bistability and fitness landscapes of antibiotic-resistant bacteria. Science 2013, 342(6162):1237435.
- 19. Friedman ND, Temkin E, Carmeli Y: The negative impact of antibiotic resistance. Clin Microbiol Infect 2016, 22(5): 416-422.
- 20. European Committee on Antimicrobial Susceptibility Testing: Breakpoint tables for interpretation of MICs and zone diameters. [http://www.eucast.org].
- Clinical and Laboratory Standards: Performance standards for antimicrobial susceptibility testing. Thirty-second edition. Wayne, PA: USA; Clinical Laboratory Standarts Institute; Supplement M100. 2022. [https://clsi.org].
- 22. Aydemir Ş, Çöplü N, Gülay Z, Gür D, Hasdemir U, Karahan ZC, Karatuna O, Kayacan ZÇ, Güner S: Antibiyotik duyarlılık testleri, EUCAST: uygulama, yorum ve uzman kurallar. Turk Mikrobiyol Cem Derg (Ek Sayı) 2016, 46: 1-204.
- 23. Türk Mikrobiyoloji Cemiyeti: TMC-ADTS kısıtlı bildirim tabloları. [https://www.tmconline.org/userfiles/file/26-37.pdf].
- 24. International Organization for Standardization: ISO 6579:2002/Amd 1 Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. Geneva, Switzerland: International Organization for Standardization; 2007.
- 25. Grimont PAD, Weill FX: Antigenic formulae of the *Salmonella* serovars, 2007. [https://www.pasteur. fr/sites/default/files/veng_0.pdf].
- 26. Clinical Laboratory Standarts Institute: Performance standards for antimicrobial susceptibility testing. 30th Edition. CLSI M100. Wayne, PA: USA; Clinical Laboratory Standarts Institute; 2020, 293.
- 27. Lowden P, Wallis C, Gee N, Hilton, A: Investigating the prevalence of *Salmonella* in dogs within the Midlands region of the United Kingdom. BMC Vet Res 2015, 11: 239.

- Wei L, Yang C, Shao W, Sun T, Wang J, Zhou Z, Chen C, Zhu A, Pan Z: Prevalence and drug resistance of *Salmonella* in dogs and cats in Xuzhou, China. J Vet Res 2020, 64(2): 263-268.
- Núñez Castro KM, Trasviña Muñoz E, García GF, Herrera Ramírez JC, López Valencia G, Medina Basulto GE, Pujol Manríquez LC, Rentería Evangelista TB: Prevalence, risk factors, and identification of *Salmonella* spp. in stray dogs of northwest Mexico. Austral J Vet Sci 2021, 51: 37-40.
- 30. Amadi VA, Hariharan H, Arya G, Matthew-Belmar V, Nicholas-Thomas R, Pinckney R, Sharma R, Johnson R: Serovars and antimicrobial resistance of non-typhoidal *Salmonella* isolated from non-diarrhoeic dogs in Grenada, West Indies. Vet Med Sci 2018, 4(1): 26-34.
- Salehi TZ, Badouei MA, Madadgar O, Ghiasi SR, Tamai IA: Shepherd dogs as a common source for *Salmonella enterica* serovar Reading in Garmsar, Iran. Turk J Vet Anim Sci 2013, 37: 102-105.
- Kiflu B, Alemayehu H, Abdurahaman M, Negash Y, Eguale T: Salmonella serotypes and their antimicrobial susceptibility in apparently healthy dogs in Addis Ababa, Ethiopia. BMC Vet Res 2017, 13(1): 134.
- 33. Teng L, Liao S, Zhou X, Jia C, Feng M, Pan H, Ma Z, Yue M: Prevalence and genomic investigation of multidrug-resistant *Salmonella* isolates from companion animals in Hangzhou, China. Antibiotics (Basel) 2022, 11(5): 625.
- 34. Jajere SM, Onyilokwu SA, Adamu NB, Atsanda NN, Saidu AS, Adamu SG, Mustapha FB: Prevalence of *Salmonella* infection in dogs in Maiduguri, northeastern Nigeria. Int J Microbiol 2014, 2014: 392548.
- 35. Frye JG, Fedorka-Cray PJ: Prevalence, distribution and characterisation of ceftiofur resistance in *Salmonella enterica* isolated from animals in the USA from 1999 to 2003. Int J Antimicrob Agents 2007, 30(2): 134-142.
- 36. Leonard EK, Pearl DL, Finley RL, Janecko N, Peregrine AS, Reid-Smith RJ, Weese, JS: Evaluation of pet-related management factors and the risk of *Salmonella* spp. carriage in pet dogs from volunteer households in Ontario (2005-2006). Zoonoses Public Health 2011, 58(2): 140-149.
- 37. Bataller E, García-Romero E, Llobat L, Lizana V, Jiménez-Trigos E: Dogs as a source of *Salmonella* spp. in apparently healthy dogs in the Valencia Region. Could it be related with intestinal lactic acid bacteria? BMC Vet Res 2020, 16(1): 268.
- Day MJ: Immune system development in the dog and cat. J Comp Pathol 2007, 137 Suppl 1:S10-5.007.
- Mondo E, Marliani G, Accorsi PA, Cocchi M, Di Leone A: Role of gut microbiota in dog and cat's health and diseases. Open Vet J 2019, 9(3): 253-258.
- Wallis CV, Lowden P, Marshall-Jones ZV, Hilton AC: Distinct fermentation and antibiotic sensitivity profiles exist in salmonellae of canine and human origin. BMC Microbiol 2018, 18(1): 15.
- Morse EV, Duncan MA, Estep DA, Riggs WA, Blackburn BO: Canine salmonellosis: A review and report of dog to child transmission of *Salmonella enteritidis*. Am J Public Health 1976, 66(1): 82-84.
- 42. Akbari Khakrizi A, Yahyaraeyat R, Ashrafi Tamai I, Beikzadeh B, Zahraei Salehi T: Prevalence assessment of *Salmonella* serovars in apparently healthy pet dogs in Tehran, Iran. Iranian J Sci Technol 2022, 14(2): 11-18.
- 43. Bianchi DM, Barzanti P, Adriano D, Martucci F, Pitti M, Ferraris C, Floris I, La Brasca R, Ligotti C, Morello S, Scardino G, Musolino N, Tramuta C, Maurella C, Decastelli L: Food

Safety Monitoring of *Salmonella* spp. in Northern Italy 2019-2021. Pathogens 2023, 12(7): 963.

- 44. Huehn S, La Ragione RM, Anjum M, Saunders M, Woodward MJ, Bunge C, Helmuth R, Hauser E, Guerra B, Beutlich J, Brisabois A, Peters T, Svensson L, Madajczak G, Litrup E, Imre A, Herrera-Leon S, Mevius D, Newell DG, Malorny B: Virulotyping and antimicrobial resistance typing of *Salmonella enterica* serovars relevant to human health in Europe. Foodborne Pathog Dis 2010, 7(5): 523-535.
- 45. Ozdemir K, Acar S: Plasmid profile and pulsed-field gel electrophoresis analysis of *Salmonella enterica* isolates from humans in Turkey. PLoS One 2014, 9(5): e95976.
- 46. Groat EF, Williams NJ, Pinchbeck G, Warner B, Simpson A, Schmidt VM: UK dogs eating raw meat diets have higher risk of *Salmonella* and antimicrobial-resistant *Escherichia coli* faecal carriage. J Small Anim Pract 2022, 63(6): 435-441.
- 47. Kizil S: Genotyping results of *Salmonella* Infantis as a food poisoning agent in Turkey between 2013 and 2017. Turk J Vet Anim Sci 2020, 44: 69-75.
- Cadirci O, Gucukoglu A, Gulel GT, Gunaydin E, Uyanik T, Kanat S: Determination and antibiotic resistance profiles of *Salmonella* serotypes isolated from poultry meat. Fresenius Environ Bull 2021, 30(04A): 4251-4261.
- Yukawa S, Uchida I, Takemitsu H, Okamoto A, Yukawa M, Ohshima S, Tamura Y: Antimicrobial resistance of *Salmonella* isolates from raw meat-based dog food in Japan. Vet Med Sci 2022, 8(3): 982-989.
- 50. Dotto G, Menandro ML, Mondin A, Martini M, Pasotto D: First detection of *Salmonella enterica* serovar Napoli in kennel dogs in Italy. Vet Rec 2017, 180(22): 544.
- 51. Yaovi AB, Sessou P, Tonouhewa ABN, Hounmanou GYM, Thomson D, Pelle R, Farougou S, Mitra A: 2022. Prevalence of antibiotic-resistant bacteria amongst dogs in Africa: A meta-analysis review. Onderstepoort J Vet Res 2022, 89(1): e1-e12.
- Walther B, Tedin K, Lübke-Becker A: Multidrug-resistant opportunistic pathogens challenging veterinary infection control. Vet Microbiol 2017, 200: 71-78.
- 53. Verma A, Carney K, Taylor M, Amsler K, Morgan J, Gruszynski K, Erol E, Carter C, Locke S, Callipare A, Shah DH: Occurrence of potentially zoonotic and cephalosporin resistant enteric bacteria among shelter dogs in the Central and South-Central Appalachia. BMC Vet Res 2021, 17(1): 313.
- 54. Hu LEH, Koizumi N, Ung TTH, Le TT, Nguyen HLK, Hoang PVM, Nguyen CN, Khong TM, Hasebe F, Haga T, Le MTQ, Hirayama K, Miura K: Antibiotic-resistant *Escherichia coli* isolated from urban rodents in Hanoi, Vietnam. J Vet Med Sci 2020, 82(5): 653-660.
- 55. Wu X, Angkititrakul S, Richards AL, Pulsrikarn C, Khaengair S, Keosengthong A, Siriwong S, Suksawat F: Risk of Antimicrobial resistant non-typhoidal *Salmonella* during asymptomatic infection passage between pet dogs and their human caregivers in Khon Kaen, Thailand. Antibiotics (Basel) 2020, 9(8): 477.

PROFIL OTPORNOSTI NA ANTIBIOTIKE KOD *SALMONELLA ENTERICA* SUBSP. *ENTERICA* IZOLOVANE IZ UZORAKA PSA I LJUDI U TURSKOJ: SLUČAJ KASTAMONU

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Salmoneloza je od velikog značaja za ljude i životinje. Kućni ljubimci, posebno psi, mogu biti asimptomatski prenosioci salmonele, pa su stoga zanemareni kao izvor salmoneloze. Oni takođe mogu širiti sojeve salmonele otporne na više lekova preko psećeg izmeta, uzrokujući probleme u lečenju salmoneloze kod ljudi. Svrha ove studije je bila da se ispita prisustvo Salmonella enterica subsp. enterica izolata iz uzorkovanog psećeg izmeta koji pripada psima koji borave u opštinskom skloništu za pse, odgajivačnice Anatolskih ovčara, i iz uzoraka krvi, stolice i zglobne tečnosti od ljudi sa simptomima gastroenteritisa, bolovima u stomaku i zglobovima. Pored toga, profili otpornosti na antibiotike kod Salmonella enterica ispitivani su izolati subsp enterica. Ukupno 45 ljudi i 11 pasa dobijeni su Salmonella enterica subsp. enterica izolati. 11 Salmonella enterica subsp. enterica dobijena od pasa identifikovana su kao S. Infantis, S. Enteritidis i S. Tiphimurium, koji odgovaraju serovarima prioritetnim za zdravlje ljudi. Utvrđeno je da su skoro svi izolati kod ljudi(42/45) i svi izolati salmonele pasa (11/11) otporni na jedan ili pet, odnosno jedan ili četiri testirana antibiotika, ali ne i na CFZ, CAZ, CST kod ljudi i CFZ, CAZ, CST, ETP u izolatima salmonele pasa. Uobičajeni profili rezistencije kod izolata salmonele poreklom od pasa i ljudi bili su GEN/AMK, AMP/GEN/CIP/ SKST, AMP/CIP, SKST. Ne treba zanemariti ispoljavanje uobičajenih profila rezistencije na antibiotike koji se inače preporučuju u lečenju salmoneloze kod ljudi. Možemo zaključiti da je neophodno praćenje kućnih ljubimaca u smislu prenošenja salmonele, kao i na eventualno širenje bakterija otpornih na antibiotike.