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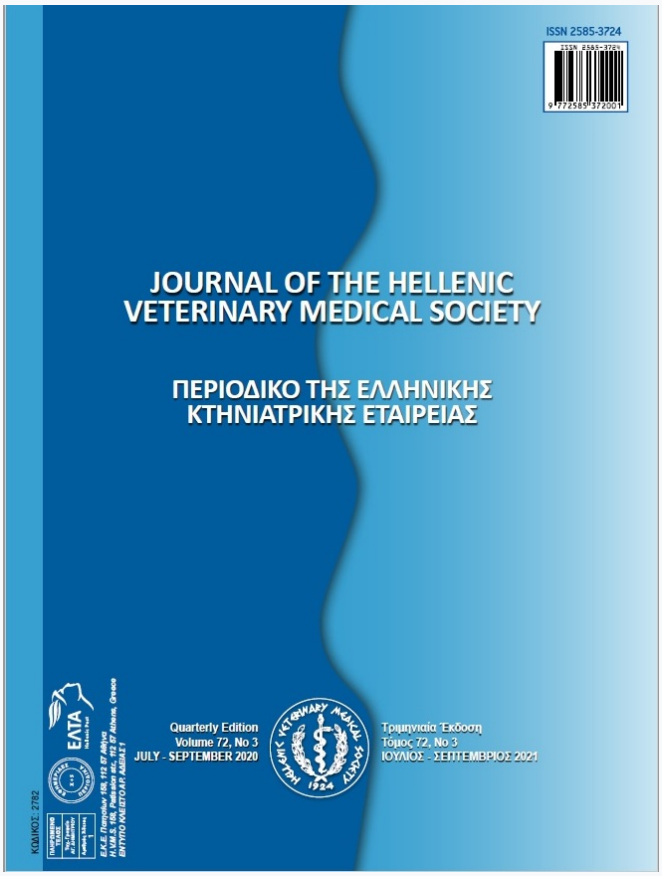

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Research article
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Differences in antimicrobial resistance of *Salmonella* spp. isolated from humans, animals and food products in Kazakhstan

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ABSTRACT: The aims of this study were twofold: 1. to isolate *Salmonella* spp. from different sources in Kazakhstan, to determine their serovars and resistance profiles and 2. to evaluate similarities in antimicrobial susceptibility of *Salmonella* spp. isolated from humans, animals and food products. From the10 212 samples tested *Salmonella* spp. were isolated in 47 cases. The predominant serovar isolated from humans and food products was *S.* Enteritidis. Although different animal species were tested the *Salmonella* spp. were isolated mainly from chickens and ducks. *S.* Enteritidis and *S.* Typhimurium were the most prevalent serovars in raw poultry meat. The most frequent resistances were those to nalidixic acid, ampicillin and tetracycline. Human isolates demonstrated lower resistance compared to animal and food isolates. The genes encoding antimicrobial resistance in human isolates in most cases were absent except for some isolates which harboured *tet*, *sul*, *stre* and *bla* TEM genes encoding resistance to the oldest antimicrobial classes. The situation in animals and food products was less beneficial as besides different genes, integrons associated with the horizontal gene transfer were detected. The findings suggest that antimicrobials in animal sector should be used more strictly, paying attention to critically important antimicrobials for humans and possible horizontal transfer of pathogens and their genetic determinants.

Keywords: *Salmonella* Paratyphi, One Health, salmonellosis, zoonoses

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INTRODUCTION

Salmonella spp. are one of the most frequently isolated foodborne pathogens. It is a major worldwide public health concern, accounting for 93.8 million foodborne illnesses and 155,000 deaths per year (Eng et al., 2015). Gastroenteritis is the most common manifestation of *Salmonella* infection, followed by bacteraemia and enteric fever (Majowicz et al. 2010). Non-typhoidal *Salmonella enterica* infection,-caused by different serovarsfrom contaminated food, is an important cause of both sporadic gastroenteritis and outbreaks internationally. In the European Union, over 91,000 human cases are reported each year (EFSA, 2018). In Australia, the incidence of infection due to *Salmonella* spp. in the community is estimated to be 185 infections per 100,000 population per year (Kirk et al., 2014). Enteric fever that is caused by *Salmonella enterica* Typhi causes illness in south-central Asia, Southeast Asia, and southern Africa by more than 100 cases per 100,000 person-years) (Marks et al., 2017). It is also prevalent in many other parts of the world. For instance, approximately 200 to 300 cases of *S.* Typhi were reported in the United States each year (Lynch et al, 2009).

Epidemiological data and incidence cases of salmonellosis are registered in many countries including the USA, EU, China, Australia, India and others while some countries still are white spots in the map according to the prevalence of salmonellosis (Eng et al., 2015). In Kazakhstan the incidence of diagnosed salmonellosis in 1999 and 2000 was about 3500-4000 cases per year, while typhoid fever incidence was about 30-110 per year (WHO 2001).

Main factors associated with *Salmonella* outbreaks include incomplete cooking of food products, improper storage and direct contact with raw ingredients (Lynch et al. 2006). The food products that are predominantly associated with the outbreaks include animal products such as milk, poultry and eggs, as well as food products such as chocolate and peanut butter (Eng et al., 2015). Animals are the main carriers and reservoir of non-typhoid *Salmonella*. The transmission of *Salmonella* usually occurs through gastrointestinal tract by ingestion of food or water contaminated with infected animals. Direct contact with infected animals also constitutes a risk of infection (Swanson et al., 2007). The worldwide incidence rate of *Salmonella* infection is high as the strains can be found naturally in the environment and in both domestic and wild animals including cats, dogs, amphibians, reptiles and rodents (Eng et al., 2015). The diversity of possible reservoirs of infection results in significant challenges for public health authorities to control the infections (Swanson et al. 2007; Dione et al. 2011).

Salmonella resistance to at least 3 antibiotics started to be reported in 1960s (Dyson et al. 2019). Since then, the frequency of resistant isolates has increased in many countries; however, the spectrum of resistance or the rate of multidrug resistant isolates depends on country, source of isolation and *Salmonella* serovar. For instance, in the USA *Salmonella* spp. recovered from poultry were resistant to streptomycin (30.9%), gentamicin (12.6%), sulfadimethoxine (20.9%), tetracycline (13.9%), and trimethoprim-sulfamethoxazole combination (8.6%). Among these isolates, 20% were multi-drug resistant, showing resistance to three or more antibiotics; noteworthy is that 67% of *S.* Heidelberg and 54% of *S.* Kentucky isolates have shown resistance to five or more antibiotics (Liljebjelke et al., 2017). In China high rates of resistant *salmonellae* from chicken were found to sulfisoxazole (76.1%), tetracycline (75.3%), ampicillin (48.0%), and ofloxacin (44.7%). Notably, antimicrobial susceptibility tests have identified resistance to polymyxin B (2.0%) and imipenem (0.5%) (Zhang et al., 2018). In Poland the resistance of salmonella isolated from non-meat food products was low with the highest prevalence to nalidixic acid (35.2%), sulphonamides (6.6%), ampicillin (4.9%), amoxicillin/clavulanic acid (2.5%), streptomycin, cefoxitin, gentamicin and tetracycline (1.6%) (Mąka et al., 2015). Although an official data about salmonellosis prevalence in Kazakhstan is collected,there is no current systematic data about the situation of antimicrobial resistance of the strains that are prevalent in humans and in other sources including animals, food products or feedstuffs. Thus, the aims of this study were twofold: 1. to isolate *Salmonella* spp. strains from different sources in Kazakhstan, to determine their serovars and resistance profiles and 2. to evaluate similarities among antimicrobial susceptibility of *Salmonella* spp. isolated from humans, animals and food products.

MATERIALS AND METHODS

Place and sampling

Bacteriological investigations were performed at the Institute of Applied Biotechnology, Kostanay University during 2018-2019. The aim was to obtain wide spectrum of *Salmonella* isolates prevalent in Ka-

zakhstan. Animal carcasses and raw meat including poultry, lamb, beefwere collected in supermarkets and markets at a retail level. Clinical and pathological material of animals was collected at slaughterhouses and animal farms. *Salmonella* spp. strains already isolated from humans (in hospitals and other health-care facilities) were delivered to the department of Microbiology of Kostanay University as isolates without the data about their concrete source (name of the patient) of isolation. Ready to use, raw and frozen food products were obtained at retail markets and supermarkets. In total 10212 samples were tested from which 1006 samples were from live animals, animal carcasses and milk, 383 food samples and 8823 human samples.

Isolation and identification of *Salmonella*

Isolation of *Salmonella* was performed according to EN ISO 6579-1 (ISO, 2017) procedure for *Salmonella* detection. Xylose Lysine Deoxycholate (XLD) agarand Salmonella Shigella (SS) Agar (Oxoid, UK) were used as plating media after the enrichment procedure. Isolates obtained from health-care facilities were re-cultivated on XLD agar.

Identification of the isolates was performed using classical biochemical testing including fermentation of carbohydrates, production of hydrogen sulfide, indole, production of lysine decarboxylase, urease, oxidase, catalase, MR-VP as well as other conventional tests.

Serotyping was performed using slide agglutination test with sera to O and H antigens (Petsal, Russia) as well as Wellcolex Colour salmonella serogroup identification latex test (Remel, UK) according to the manufacturer's instructions.

Susceptibility testing

Antimicrobial susceptibility testing was performed using the disc diffusion method according to Kirby-Bauer (Hudzicki, 2009). The following discs with antimicrobials (μg) (Thermo Fisher, UK) were used: ampicillin (10), cefoperazone (75), cefoxitin (30), streptomycin (10), kanamycin (30), gentamicin (10), tetracycline (30), doxycycline (30), chloramphenicol (30), ciprofloxacin (5), nalidixic acid (30), gemifloxacin (5), enrofloxacin (5), ofloxacin (5) and sulfamethoxazole-trimethoprim (25). Interpretation of the results was carried out using CLSI clinical breakpoints (CLSI, 2019) set for *Salmonella* except for ofloxacin, gemifloxacin and nalidixic acid for which breakpoints set for Enterobacteriaceae were used.

Detection of the genes encoding antimicrobial resistance

Molecular testing of the isolates was performed at the Microbiology and Virology Institute, Lithuanian University of Health Sciences. DNA material for molecular testing was obtained after bacterial lysis according to the extraction protocol prepared by the EU Community Reference Laboratory for Antimicrobial Resistance with slight modifications (Ruzauskas et al., 2014). Detection of the genes encoding antimicrobial resistance was performed by PCR. Annealing temperatures and oligonucleotides used are presented in Table 1.

Table 1. Antimicrobial resistance genes tested and oligonucleotide primers used in the study

Primer name	Sequencece(5'-3')	Size, bp and t (°C)	Target gene	Source
*bla*TEM-F	GAGTATTCAACATTTTCGT	857 (50)	*tem*	Maynard et al., 2003
*bla*TEM-R	ACCAATGCTTAATCAGTGA			
*bla*SHV-F	TCGCCTGTGTATTATCTCCC	768 (60)	*shv*	Ojdana et al., 2014
*bla*SHV-R	CGCAGATAAATCACCACAATG			
*oxa*1-F	TCAACAAATCGCCAGAGAAG	276 (55)	*oxa* group I	Bert et al., 2002
*oxa*1-R	TCCCACACCAGAAAAACCAG			
*oxa*group 5-F	AGCCGCATATTTAGTTCTAG	644(56)	*oxa* group V	Bert et al., 2002
*oxa*group 5-R	ACCTCAGTTCCTTTCTCTAC			
*ctx*M-F	ATGTGCAGYACCAGTAARGT	593 (50)	*ctxM*	Pagani et al., 2003
*ctx*M-R	TGGGTRAARTARGTSACCAGA			
ctxM group 2-F	ATGATGACTCAGAGCATTCGCCGC	876 (56)	*ctxM2*	Celenza et al., 2006
ctcM group 2-R	TCAGAAACCGTGGGTTACGATTTT			
*cmy*2-F	GCACTTAGCCACCTATACGGCAG	758 (58)	*cmy*	Hasman et al., 2005
*cmy*2-R	GCTTTTCAAGAATGCGCCAGG			

PER-1-F	ATGAATGTCATTATAAAAGCT	927 (48)	*per*	Celenza et al., 2006
PER-1-R	TTAATTTGGGCTTAGGG			
PER-2-F	ATGAATGTCATCACAAAATG	927 (49)		Celenza et al., 2006
PER-2-R	TCAATCCGGACTCACT			
*tet*A-F	GTGAAACCCAACATACCCC	888 (55)	*tet*A	Maynard et al., 2003
*tet*A-R	GAAGGCAAGCAGGATGTAG			
*tet*B-F	CCTTATCATGCCAGTCTTGC	774 (55)	*tet*B	Maynard et al., 2003
*tet*B-R	ACTGCCGTTTTTTCGCC			
aadB-F	ATGGACACAACGCAGGTCGC	534 (55)	*aad*B	Asadollahi et al., 2012
aadB-R	TTAGGCCGCATATCGCGACC			
*aad*A-F	GTGGATGGCGGCCTGAAGCC	528 (68)	*aad*A	Asadollahi et al., 2012
*aad*A-R	AATGCCCAGTCGGCAGCG			
*rmt*B-F	ATGAACATCAACGATGCCCT	769 (55)	*rmt*B	Yan et al., 2004
*rmt*B-R	CCTTCTGATTGGCTTATCCA			
*arm*A-F	CAAATGGATAAGAATGATGTT	774 (55)	*arm*A	Galimand et al., 2003
*arm*A-R	TTATTTCTGAAATCCACT			
*aph*A1-F	AAACGTCTTGCTCGAGGC	500 (55)	*aph*A1	Frana et al., 2001
*aphA*1-R	CAAACCGTTATTCATTCGTGA			
*aacA*4-F	ATGACTGAGCATGACCTTGCG	487 (55)	*aacA4*	Odumosu et al., 2015
*aacA*4-R	TTAGGCATCACTGCGTGTTCG			
aac(3)II-F	TGAAACGCTGACGGAGCCTC	369 (65)	*aac(3)II*	Sandvang et al., 2009
aac(3)II-R	GTCGAACAG GTAGCACTGAG			
*str*A-F	CCTGGTGATAACGGCAATTC	546 (55)	*str*A	Lanz et al., 2003
*str*A-R	CCAATCGCAGATAGAAGGC			
*str*B-F	ATCGTCAAGGGATTGAAACC	509 (55)	*str*B	Lanz et al., 2003
*str*B-R	GGATCGTAGAACATATTGGC			
*cat*II-F	ACACTTTGCCCTTTATCGTC	495 (55)	*cat*II	Vassort-Bruneau et al., 1996
*cat*II-R	TGAAAGCCATCACATACTGC			
*cml*A-F	TTGCAACAGTACGTGACAT	293 (55)	*cml*A	Keyes et al., 2000
*cml*A-R	ACACAACGTGTACAACCAG			
*sul*1-F	TTCGGCATTCTGAATCTCAC	822 (55)	*sul*1-F	Christabel et al., 2012
*sul*1-R	ATGATCTAACCCTCGGTCTC			
*sul*2-F	CGGCATCGTCAACATAACC	722 (50)	*sul*2-F	Pereten et al., 2003
*sul*2-R	GTGTGCGGATGAAGTCAG			
*sul*3-F	GAGCAAGATTTTTGGAATCG	792 (51)	*sul*3-F	Pereten et al., 2003
*sul*3-R	CATCTGCAGCTAACCTAGGGCTTTGA			
dfr1-F	ACGGATCCTGGCTGTTGGTTGGACGC	254 (55)	*dfr*1	Gibreel et al., 1998
dfr1-R	CGGAATTCACCTTCCGGCTCGATGTC			
dfr5-F	GCBAAAGGDGARCAGCT	394 (44)	*dfr*5	Seputiene et al., 2010
dfr5-R	TTTMCCAYATTTGATAGC			
dfrA7-F	AAAATTTCATTGATTTCTGCA	471 (44)	*dfr*7	Navia et al., 2003
dfrA7-R	TTAGCCTTTTTTCCAAATCT			
mcr1-F	CGGTCAGTCCGTTTGTTC	309 (58)	*mcr1*	Liu et al, 2015
mcr1-R	CTTGGTCGGTCTGTAGGG			
mcr2-F	TGTTGCTTGTGCCGATTGGA	567 (58)	mcr2	Xavier et al., 2016
mcr2-R	AGATGGTATTGTTGGTTGCTG			
qnrA-F	ATTTCTCACGCCAGGATTTG	516 (53)	qnrA	Robicsek et al., 2006
qnrA-R	GATCGGCAAAGGTTAGGTCA			
qnrB-F	GATCGTGAAAGCCAGAAAGG	469 (53)	qnrB	Robicsek et al., 2006
qnrB-R	ACGATGCCTGGTAGTTGTCC			
qnrS-F	ACGACATTCGTCAACTGCAA	417 (53)	qnrS	Robicsek et al., 2006
qnrS-R	TAAATTGGCACCCTGTAGGC			
qepA-F	CAGTGGACATAAGCCTGTTC	218 (60)	qepA	Liu et al., 2008
qepA-R	CCCGAGGCATAGACTGTA			
teg1-F	TTATTGCTGGGATTAGGC	164 (55)	integrase 1 class	Chen et al., 2013
teg1-R	ACGGCTACCCTCTGTTATC			
teg2-F	ACGACATTCGTCAACTGCAA	233 (50)	integrase II class	Goldstein, 2001
teg2-R	TAAATTGGCACCCTGTAGGC			

Table 2. Characteristics of *Salmonella* isolated from humans in Kazakhstan

Isolate number	Serovar	Phenotypic Resistance	Genotypic Resistance	Integrons
733	*S.* Tshiongwe	CIP		-
735	*S.* Enteritidis	NAL, CIP		-
737	*S.* Blegdam			-
739	*S.* Blegdam			-
990	*S.* Enteritidis	NAL, ENR		-
991	*S.* Enteritidis	CIP		-
993	*S.* Enteritidis	TET		-
994	*S.* Enteritidis	STR, DOX	*strB*	-
998	*S.* Enteritidis	ENR, NAL, CIP		-
999	*S.* Enteritidis	NAL, ENR, CIP, GEMI,		-
1106	*S.* Enteritidis	NAL, CIP		-
1107	*S.* Enteritidis			-
1108	*S.* Enteritidis			-
1109	*S.* Enteritidis			-
1110	*S.* Enteritidis			-
1111	*S.* Enteritidis	NAL, CIP		-
1112	*S.* Enteritidis			-
1113	*S.* Enteritidis	TET	*tet B*	-
1205	*S.* Enteritidis	NAL, ENR, CIP, GEMI		-
1206	*S.* Enteritidis	AMP, TET, DOX, NAL, ENR, CIP, OFX, SXT	*blaTEM, tet A, tet B, sul3*	+ (class I)
1280	IIIc 4p	NAL, ENR, CIP		-

NAL - nalidixic acid; CIP - ciprofloxacin; ENR - enrofloxacin; TET - tetracycline, DOX - doxycycline; GEMI - gemifloxacin; AMP - ampicillin; OFX - ofloxacin; STRE - streptomycin; SXT - sulfamethoxazole/trimethoprim

Table 3. Characteristics of *Salmonella* isolated from animals in Kazakhstan

Serovar	Source of Isolation	Phenotypic Resistance	Genotypic resistance	Integrons
*S.*Typhimurium	duck	STR, KAN, ENR		-
*S.*Typhimurium	duck	STR, CHL, TET, ENR,		-
S. Typhimurium	chicken			-
S. Typhimurium	chicken			-
S. Paratyphi C	chicken	CHL, TET, DOX, NAL, CIP, SXT	*tetA, sul3, cmlA, catII*	+ (class I)
*S.*Enteritidis	chicken	NAL, CIP		-
*S.*Enteritidis	chicken	NAL, CIP		-
*S.*Enteritidis	chicken	AMP, STR, SXT	*Bla TEM, aadA, dfr1*	-
*S.*Enteritidis	chicken	STR, TET, DOX, ENR, CIP, GEMI, NAL, SXT	*tet A, sul 2, dfr1, strA, str B*	-
*S.*Enteritidis	chicken	ENR, NAL, CIP		-
*S.*Enteritidis	chicken	AMP, STR, ENR	*bla TEM, aacA4,*	-
*S.*Enteritidis	chicken	NAL, ENR, CIP		-
*S.*Moscow	chicken	NAL, ENR, CIP		-
S. Paratyphi C	chicken (imported)	FOX, STR, TET, DOX, NAL, ENR, CIP, OFX, GEMI	*aadA, tet A, qnr B*	+ (class I)

NAL - nalidixic acid; CIP - ciprofloxacin; ENR - enrofloxacin; TET - tetracycline, DOX - doxycycline; GEMI - gemifloxacin; AMP - ampicillin; OFX - ofloxacin; SXT - sulfamethoxazole/trimethoprim; CHL - chloramphenicol; STR - streptomycin; KAN - kanamycin

Table 4. Characteristics of *Salmonella* isolated from food products in Kazakhstan

Serovar	Source of isolation	Phenotypic Resistance	Genotypic resistance	Integrons
S. Enteritidis	Roasted chicken drumstics			-
S. Enteritidis	Roasted chicken drumstics	NAL, ENR, CIP		-
S. Enteritidis	Sausage	NAL, ENR, CIP		-
S. Enteritidis	Cake			-
S. Enteritidis	Ice-cream	NAL, ENR, CIP		-
S. Enteritidis	Cake	NAL, ENR, CIP		-
S. Enteritidis	cake		tet B	-
S. Enteritidis	Refrigerated chicken drumstics	AMP, STR, KAN, TET, DOX, ENR		-
S. Enteritidis	Sausage	AMP, STR, KAN, CHL, DOX, ENR		-
S. Enteritidis	Refrigerated chicken drumstics	AMP,STR, KAN, CN, TET, DOX		-
Salmonella group C	Force meat	AMP, STR, CHL, TET, DOX, NAL, ENR, CIP, OFX, GEMI, SXT	aadB, tet A, tet B, sul3, catII, qnrA	+ (Class I)
S. Tennessee	Meat in paste	AMP, CFP, KAN, CHL, TET, DOX, NAL, ENR, CIP, SXT	ctxM, aphA1, aadA, tet A, sul3, cmlA, catII	+ (ClassI, Class II)

NAL - nalidixic acid; CIP - ciprofloxacin; ENR - enrofloxacin; TET - tetracycline, DOX - doxycycline; GEMI - gemifloxacin; AMP - ampicillin; CN - gentamicin; OFX - ofloxacin; SXT - sulfamethoxazole/trimethoprim; CHL - chloramphenicol; STR - streptomycin; KAN - kanamycin; CFP - cefpodoxime

Data analysis

Occurrence of multi-resistant isolates was calculated by dividing the number of isolates resistant to at least 3 antimicrobial classes by the total number of obtained isolates. Statistical analysis was performed using IBM SPSS Statistics package, version 20.

Comparison between categorical variables was calculated using the chi-square test. Results were considered statistically significant if $P \leq 0.05$.

RESULTS

From total 10 212 samples tested (1006 samples from live animals, animal carcasses and milk; 383 food and 8823 human samples)*Salmonella* were isolated in 47 cases (0.46%). Twenty one (21) isolates (0.24%) were obtained from humans, fourteen (14) (1.39%) - from live or slaughtered animals and twelve (12) isolates (3.13 %) - from ready to use, raw or refrigerated food products (Tables 2-4).

As it could be seen from Table 2, the predominant *Salmonella* serovar isolated from humans was *S.* Enteritidis (81% from all isolates). Susceptibility varied among the strains: seven isolates were susceptible to all tested antimicrobials while a single isolate was resistant to eight (8) of the tested antimicrobials. The most frequent resistance was towards nalidixic acid, fluoroquinolones and tetracyclines. The prevalence of the genes encoding resistance was low. Integrons were detected in a single multi-resistant isolate of *S.* Enteritidis.

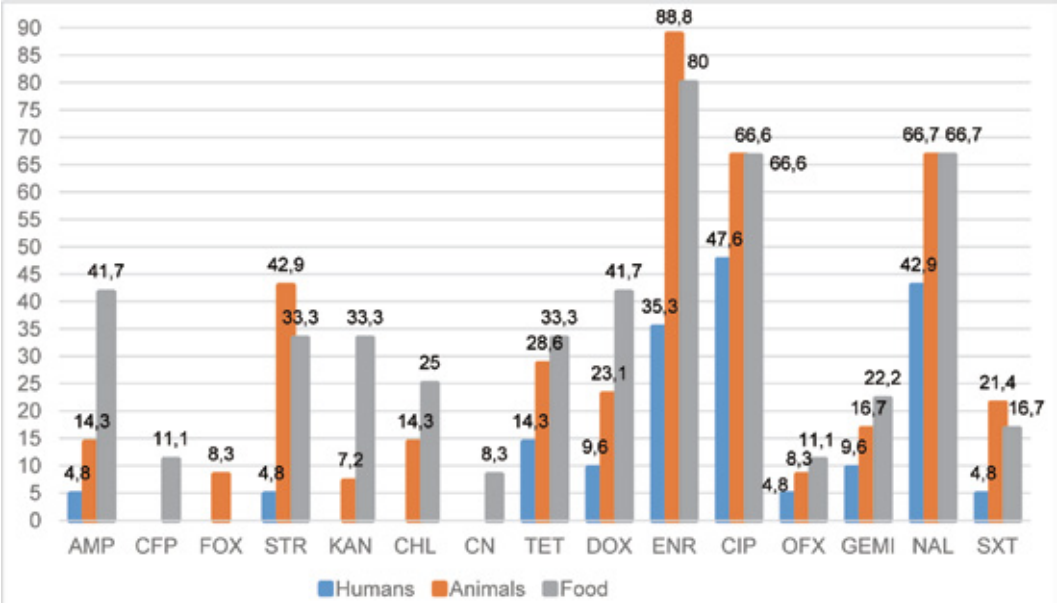
Although different animal species were tested the *Salmonella* were isolated only from chickens and ducks (Table 3). The serovar variety in poultry was higher to those of humans. The predominant serovar were *S.* Typhimurium and *S.* Enteritidis. Two isolates were identified as *S.* Paratyphi C. One of those isolates was resistant to tetracyclines, nalidixic acid, ciprofloxacin, chloramphenicol, sulfonamides and trimethoprim and harboured class I integrons while the other - to 2d generation cephalosporins, streptomycin, tetracyclines and all fluoroquinolones tested. Two (2) strains out of fourteen (14) isolated from animals were susceptible to all antimicrobials tested while six (6) isolates were multi-resistant.

S. Enteritidis was the predominant serovarin food products (81% from all isolates) however, two other serovars- (*Salmonella*group C and *S.* Tennessee) had much wider spectrum of resistance. Five (5) isolates out of twelve (12)of food origin were multi-resistant, two (2) strains were susceptible to all antimicrobials and four (4) had similar resistance patterns being resistant only to (fluoro)quinolones. The isolate of *S.* Tennessee was a single strain harboured integrons of both I and II class.

The frequency of antimicrobial resistance in *Sal-*

monella isolates obtained from different sources is presented in Fig 1.

The most frequent resistance among *Salmonella* isolates was demonstrated to fluoro(quinolones), ampicillin, streptomycin and tetracycline. Resistance to critically important antimicrobials for humans was also detected and varied depending on the source of isolation. Human isolates demonstrated lower resistance compared to animal and food isolates. Food isolates were more frequently resistant to antimicrobials compared to animal isolates except for streptomycin and enrofloxacin which were more frequently non effective against animal isolates and for nalidixic acid for which the number of resistant isolates from animals and foods had the same range. The correlation between the strains isolated from different sources regarding their resistances to separate antimicrobials is presented in Table 5.



NAL - nalidixic acid; CIP - ciprofloxacin; ENR - enrofloxacin; TET - tetracycline, DOX - doxycycline; GEMI - gemifloxacin; AMP - ampicillin; CN - gentamicin; OFX - ofloxacin; SXT - sulfamethoxazole/trimethoprim; CHL - chloramphenicol; STR - streptomycin; KAN - kanamycin; FOX - cefoxitin; CFP - cefpodoxime

Fig 1. Antimicrobial resistance (% of resistant isolates) of *Salmonella* isolated from humans, animals and food products

Table 5. Statistical differences among resistances in *Salmonella* isolates from different sources

Antibiotics	Human vs Animals			Human vs Food			Animals vs Food		
	Value	df	P - value	Value	df	P - value	Value	df	P - value
Ampicillin	0.972	1	0.324	6.991	1	**0.008**	2.462	1	0.117
Cefoperazone	constant			2.414	1	0.120	1.400	1	0.237
Cefoxitin	1.805	1	0.179	constant			0.788	1	0.375
Streptomycin	10.862	1	**0.001**	7.966	1	**0.005**	0.248	1	0.619
Kanamycin	1.544	1	0.214	7.966	1	**0.005**	2.854	1	0.091
Gentamicin	constant			1.805	1	0.179	1.213	1	0.271
Levomycetin	3.182	1	0.074	5.775	1	**0.016**	0.478	1	0.490
Tetracycline	2.146	1	0.143	2.910	1	0.088	0.069	1	0.793
Doxycycline	1.176	1	0.278	4.721	1	**0.030**	0.991	1	0.319
Enrofloxacin	6.801	1	**0.009**	5.040	1	**0.025**	0.281	1	0.596
Ciprofloxacin	0.638	1	0.424	0.524	1	0.469	0.000	1	1.000
Ofloxacin	0.171	1	0.679	0.408	1	0.523	0.046	1	0.830
Gemifloxacin	0.366	1	0.545	0.879	1	0.348	0.103	1	0.748
Nalidixic acid	1.733	1	0.188	1.429	1	0.232	0.000	1	1.000
Sulfamethoxazole/ trimethoprim	2.305	1	0.129	1.310	1	0.252	0.094	1	0.759

DISCUSSION

This study describes the prevalence of *Salmonella* spp. in different sources in Kazakhstan. Up to date only the data from humans were collected by national and international responsible institutions within the country. Salmonellosis is one of the leading causes of foodborne infections in humans with animals being reservoirs of *Salmonella*. *Salmonella* colonizes the gastrointestinal tract of food animals (Andino et al., 2015) and is shed via feces (Narváez-Bravo et al., 2013). Although the clinical manifestation of salmonellosis in different food-producing animal species can be unequally expressed, all animal species can be a source of foodborne infection (Chaney et al., 2017; Evangelopoulou et al., 2015; Pande et al., 2016).

Knowledge ofthe prevalence and diversity of *Salmonella* serovars in animals and food can provide important information necessary to develop preventive measures and strategies at different stages of the food chain such as application hazard analysis and critical control programs in meat production industries to ensure food safety (Tietjen and Fung, 1995; Gutema et al., 2019). According to this study the most prevalent serovar of *Salmonella* in Kazakhstan was *S.* Enteritidis which was the most frequently detected both in humans (81%) and food products (83%). According to the World Health Organization, *S.* Enteritidis is one of the most frequently isolated *Salmonella* serovars (along with *S.* Typhimurium) from countries involved in the Global Foodborne Infections Network (Hendriksen et al., 2011). The other serovars isolated from humans included *S.* Blegdam and *S.* Tshiongwe. *S.* Blegdam which is genetically similar to *S.* Enteritidis was isolated for the first time in the USA in the middle of last century from a patient suffering with the enteric type of fever and extensive erythema during the fifth week after onset (Holt and Newton, 1948). There is a lack of data about its prevalence during past decades, although *S.* Blegdam was recently isolated from broilers in Egypt (Ammar et al., 2019). *S.* Tshiongwe was described as a pathogenic serovar for both humans and animals and was also isolated from foods (Thong et al., 2004; Mshelbwala et al., 2017). This study demonstrated that the variety of serovars prevalent in animals in Kazakhstan was not wide; besides *S.* Enteritidis, which prevalence was 50% of all serovars, *S.* Typhimurium (29%) was also common. This serovar was recovered from poultry. The disease caused by *S.* Typhimurium is of public health significance, as it is associated with food poisoning in humans (Dar et al., 2017). The other highly pathogenic to man serovar-*S.* Paratyphi C was isolated from chicken carcasses in two cases. This serovaris associated with human disease although the carriers can be different animal species (ECDC, 2019). Although paratyphoid fever is a rare disease in Europe and the USA it is still prevalent in Asia (Ekdahl et al., 2005).

Interesting data was also obtained according to the antimicrobial susceptibility testing: the isolates from humans were less frequently resistant to different antimicrobials comparing to animal and particularly food isolates, although it is known that salmonellosis is a foodborne human infection. All of the isolates obtained from humans, *in vitro* were susceptible to chloramphenicol, cephalosporins and aminoglycosides (except one isolate which was resistant to streptomycin), while the isolates from animals and foods had different susceptibility patterns. Although aminoglycosides are poorly effective against salmonellosis *in vivo* (Kihlstrom and Andaker, 1985), these antimicrobials are good indicators testing *in vitro* when comparing differences in antimicrobial susceptibility of different isolates, particularly isolated from different sectors. As it is important to understand the chain of salmonellosis “from farm to table” the data obtained in this study is interestingly enough demonstrating quite big differences among susceptibility in salmonella isolates from animals, foods and humans. The most frequent resistance in the isolates irrespective from the source of isolation was towards (fluoro) quinolones, aminopenicillins, streptomycin, tetracyclines and sulphamethoxazole/trimethoprim. These antimicrobials are frequently used both in humans and animals. Comparing the resistance between animal and food isolates the differences were less obvious except for beta-lactams and kanamycin with the highest resistance frequency being in food isolates. Isolates from animals had the highest resistance towards enrofloxacin compared to isolates from other sources. This is probably associated with the frequent use of enrofloxacin for animal treatment in Kazakhstan as it is known that broad use of fluoroquinolones has been followed by emergence of resistance to this class of antimicrobials (Hooper, 2001). Although all food products from which *Salmonella* were isolated contained ingredients of animal origin (meat, milk products or eggs) only a part of the products were originated from Kazakhstan while some of them were imported (data are not presented). Resistance to 3d generation cephalosporins was detected only in the isolates from foods but not from humans and animals, meaning that *salmonellae* circulating in Kazakh-

stan are quite specific and isolated. This fact is also proved by the low overall prevalence of *Salmonella* and by the poor variety of serovars circulating within the country. Imported food however, may change the situation in case of foodborne infections appear. The current situation regarding antimicrobial resistance in humans is beneficial with possibility to treat salmonellosis with antimicrobials of the 1st choice. The best known genes encoding antimicrobial resistance in human isolates mostly were absent except for some isolates which harboured *tet*, *sul*, *stre* and *bla*TEM genes encoding resistance to the oldest antibiotic classes. The situation in animals and food products was less beneficial as besides different genes, integrons associated with the horizontal gene transfer were detected more frequently. These findings suggest that antimicrobials in animal sector should be used more strictly particularly paying attention to critically important antimicrobials for humans and possible horizontal transfer of pathogens and their genetic determinants between environment (animals, food ingredients) and humans. Since multi-resistant highly pathogenic to humans serovars,as *S*. Paratyphi C, were detected in animal carcasses, monitoring of *Salmonella* prevalence in animals should be implemented on a regular basis and measures for *Salmonella* eradication should be foreseen.

In conclusion, food import is one of the key factors changing the *Salmonella* variety and its antimicrobial resistance patterns in Kazakhstan. Usage of enrofloxacin in veterinary medicine however, is the factor of increasing salmonella resistance to fluoroquinolones that may have negative impact on treatment of salmonellosis infections.

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CONFLICT OF INTERST

The authors declare no conflict of interest.

REFERENCES

Ammar AM, Abdeen EE, Abo-Shauma UP, Fekry E, Kotb Elmahallawy E (2019) Molecular characterization of virulence and antibiotic resistance genes among Salmonella serovars isolated from broilers in Egypt. Lett Appl Microbiol68:188-195.

Andino A, Hanning I (2015)*Salmonella enterica*: survival, colonization, and virulence differences among serovars. Sci World J 2015:520179.

AsadollahiP, AkbariM, Soroush S, Taherikalani M, Asadollahi K, Sayehmiri K (2012) Antimicrobial resistance patterns and their encoding genes among *Acinetobacter baumannii* strains isolated from burned patients. Clin Infect Dis38:1198-1203.

Bert F, Branger C, Lambert-Zechovsky N (2002) Identification of PSE and OXA β-lactamase genes in *Pseudomonas aeruginosa* using PCR-restriction fragment length polymorphism. J Antimicrob Chemother 50:11-18.

Celenza G, Pellegrini C, Caccamo M, Segatore B, Amicosante G, Perilli M (2006) Spread of *bla*CTX-M-type and *bla*PER-2 β-lactamase genes in clinical isolates from Bolivian hospitals. J Antimicrob Chemother 57:975-978.

Chaney WE, Agga GE, Nguyen SV, Arthur TM, Bosilevac JM, Dreyling E, Rishi A, Brichta-Harhay D (2017) Rapid detection and classification of *Salmonella enterica* shedding in feedlot cattle utilizing the roka bioscience atlas *Salmonella* detection assay for the analysis of rectoanal mucosal swabs. J Food Prot80:1760-1767.

Chen CH, Huang CC (2013)Risk factor analysis for extended-spectrum β-lactamase-producing *Enterobacter cloacae* bloodstream infections in central Taiwan. BMC Infect Dis 13:417.

Christabel M, Budambula N, KiiruJ, Kariuki S (2012) Characterization of antibiotic resistance in environmental enteric pathogens from Kibera slum in Nairobi-Kenya. Afr J Bacteriol Res4:46-54.

CLSI (2019) Performance Standards for Antimicrobial Susceptibility Testing. 29th ed. CLSI supplement M100. Wayne, P. A.: Clinical and Laboratory Standards Institute.

DarMA, AhmadSM, Bhat SA, Ahmed R, Urwat U, MumtazPT, Bhat SA, Dar TA, Shah RA, Ganai NA (2017) *Salmonella typhimurium* in poultry: a review. World Poultry Sci J 73:345-354.

DioneMM, IkumapayiUN, SahaD, MohammedNI, GeertsS, IevenM, AdegbolaRA, AntonioM (2011)Clonal differences between non-typhoidal *Salmonella* (NTS) recovered from children and animals living in close contact in the Gambia. PLoS Negl Trop Dis5:e1148.

Dyson ZA, Klemm EJ, Palmer S, Dougan G (2019) Antibiotic resistance and typhoid, Clin Infect Dis 68 :165-170.

Ekdahl K, de Jong B, Wollin R, Andersson Y (2005)Travel-associated non-typhoidal salmonellosis - geaographical and seasonal differences and serotype distribution. Clin Microbiol Infect 11:138-144.

Eng SK, Pusparajah P, Ab Mutalib NS, Ser HL, Chan KG, Lee,LH (2015) *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance.Front Life Sci8:284-293.

European Centre for Disease Prevention and Control. Facts about typhoid and paratyphoid fever. Interactive https://www.ecdc.europa.eu/en/typhoid-and-paratyphoid-fever/facts. Accsessed: 26-03-2020.

Evangelopoulou G, Kritas S, Christodoulopoulos G, Burriel A R (2015)The commercial impact of pig *Salmonella* spp. infections in border-free markets during an economic recession. Vet World 8:257-272.

Frana TS, CarlsonSA, Griffith RW (2001) Relative distribution and conservation of genes encoding aminoglycoside-modifying enzymes in *Salmonella enterica* serotype Typhimurium phage type DT104. Appl Environ Microbiol 67:445-448.

Galimand M, Courvalin P, Lambert T (2003) Plasmid-Mediated High-Level Resistance to Aminoglycosides in *Enterobacteriaceae* Due to 16S rRNA Methylation. Antimicrob Agents Chemother47:2565-2571.

Gibreel A, Sköld O (1998)High-level resistance to trimethoprim in clinical isolates of *Campylobacter jejuni* by acquisition of foreign genes (*dfr1* and *dfr9*) expressing drug-insensitive dihydrofolate reductases. Antimicrob Agents Chemother 42:3059-3064.

Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, Grady M, Liebert C, Summers AO, White DG, Maurer JJ (2001) Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob Agents Chemother45:723-726.

Gutema FD, Agga GE, Abdi RD, De Zutter L, Duchateau L,Gabriël S (2019) Prevalence and serotype diversity of *Salmonella* in apparently healthy

cattle: Systematic review and meta-analysis of published studies, 2000-2017. Front Vet Sci6:102.

Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM (2005) β-lactamases among extended-spectrum β-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. J Antimicrob Chemother 56:115-121.

Hendriksen RS, Vieira AR, Karlsmose S, Lo Fo Wong DM, Jensen AB, Wegener HC, Aarestrup FM (2011) Global monitoring of *Salmonella* serovar distribution from the World Health Organization global foodborne infections network country data bank: results of quality assured laboratories from 2001 to 2007. Foodborne Pathog Dis 8:887-900.

Holt RA, Newton H (1948) The occurrence of *Salmonella blegdam* in Louisiana. J Lab Clin Med 33:1115-1158.

Hooper DC (2001) Emerging mechanisms of fluoroquinolone resistance. Emerg Infect Dis 7:337-341.

Hudzicki J (2009) Kirby-Bauer disk diffusion susceptibility test protocol. ASM Microbe Libr, URL (https://www.asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf). (accessed 26.03.2020).

ISO 6579-1:2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Horizontal method for the detection of *Salmonella* spp. International Organization for Standardization, Geneva, Switzerland.

Keyes K, Hudson C, Maurer JJ, Thayer S, White DG, Lee MD (2000) Detection of florfenicol resistance genes in *Escherichia coli* isolated from sick chickens. Antimicrob Agents Chemother44:421-424.

Kihlstrom E, Andaker L (1985)Inability of gentamicin and fosfomycin to eliminate intracellular Enterobacteriaceae. J Antimicrob Chemother 15:723-728.

Kirk M, Ford L, Glass K, Hall G (2014) Foodborne illness, Australia, circa 2000 and circa 2010. Emerg Infect Dis 20:1857-1864.

Lanz R, Kuhnert P, Boerlin P (2003) Antimicrobial resistance and resistance genes determinants in clinical *Escherichia coli* from different animal species in Switzerland. Vet Microbiol91:73-84.

Liljebjelke KA, Hofacre CL, White DG, Ayers S, Lee MD, Maurer JJ (2017) Diversity of antimicrobial resistance phenotypes in *Salmonella* isolated from commercial poultry farms. Front Vet Sci 4:96.

Liu JH, Deng YT, Zeng ZL, Gao JH, Chen L, Arakawa Y,Chen ZL (2008) Coprevalence of plasmid-mediated quinolone resistance determinants QepA, Qnr, and AAC(6′)-Ib-cr among 16S rRNA methylase RmtB-producing *Escherichia coli* isolates from pigs. AntimicrobAgents Chemother 52:2992-2993.

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu, JH, Shen J (2015) Emergence of plasmid mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis, S1473-3099(15)00424-7.

Lynch MF, Blanton EM, Bulens S, Polyak C, Vojdani J, Stevenson J, Medalla F, Barzilay E, Joyce, K, Barrett T, Mintz ED (2009) Typhoid fever in the United States, 1999-2006. JAMA 302:859-865.

LynchM, PainterJ, WoodruffR, BradenC (2006)Surveillance for foodborne-disease outbreaks - United States, 1998-2002. MMWR Surveill Summ10:1-42.

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra R M (2010) The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis 50:882-889.

Mąkał, Maćkiw E, Ścieżyńska H, Popowska M (2015)Occurrence and antimicrobial resistance of *Salmonella* spp. isolated from food other than meat in Poland.Ann Agric Environ Med AAEM22:403-408.

Marks F, von Kalckreuth V, Aaby P, Adu-Sarkodie Y, El Tayeb MA, Ali M, Aseffa A, Baker S, Biggs HM, Bjerregaard-Andersen M, et al. (2017) Incidence of invasive salmonella disease in sub-Saharan Africa: a multicentre population-based surveillance study. Lancet Glob Health 5:e310.

Maynard C, Fairbrother JM, Bekal S, Sanschagrin F, Levesque RC, Brousseau R, Masson L, Larivière S,Harel J (2003) Antimicrobial resistance genes in enterotoxigenic Escherichia coli O149:K91 isolates obtained over a 23-year period from pigs. Antimicrob Agents Chemother 47:3214-3221.

Mshelbwala FM, Ibrahim NDG, Saidu SNA, Azeez AA, Akinduti PA, Kwanashie CN, Kadiri AKF, Muhammed M, Fagdamila IO, Luka PD (2017) Motile Salmonella serotypes causing high mortality in poultry farms in three Sauthwestern States of Nigeria. Vet Rec Open 4:e000247.

Narváez-Bravo C, Rodas-González A, Fuenmayor Y, Flores-Rondon C, Carruyo G, Moreno M, Perozo-Mena A, Hoet AE (2013)*Salmonella*. on feces, hides and carcasses in beef slaughter facilities in Venezuela. Int J Food Microbiol 166:226-230.

Navia MM, Ruiz J, Cespedes SJ, Vila J (2003)Detection of dihydrofolate reductase genes by PCR and RFLP. Diagn Microbiol Infect Dis 46:295-298.

Odumosu BT,Akintimehin AR (2015)Occurrence of extended-spectrum beta-lactamase producing *Enterobacteriaceae* isolates in communal water sources in Ogun State, Nigeria.Afr J Clin Exp Microbiol16:28-32.

Ojdana D, Sacha PB, Wieczorek P, Czaban B, Michalska A, Jaworowska J, Jurczak A, Poniatowski B, Tryniszewska E (2014)The occurrence of *bla*CTX-M, *bla*SHV, and *bla*TEM genes in extended-spectrum *β*-Lactamase-positive strains of *Klebsiellapneumoniae*, *Escherichia coli*, and *Proteus mirabilis* in Poland. Int J Antibiot :93584.

Pagani L, Dell'Amico E, Migliavacca R, D'Andrea MM, Giacobone E, Amicosante GER, Rossolini GM (2003)Multiple CTX-M-Type extended-spectrum β-lactamases in nosocomial isolates of *Enterobacteriaceae* from a hospital in northern Italy. J Clin Microbiol 41:4264-4269.

Pande VV, Devon RL, Sharma P, McWhorter AR, Chousalkar KK (2016) Study of *Salmonellatyphimurium* infection in laying hens. Front Microbiol7:203.

PerretenV, BoerlinPA (2003) New sulfonamide resistance gene (*sul3*) in *Escherichia coli* is widespread in the pig population of Switzerland. Antimicrob Agents Chemother47:1169-1172.

Robicsek A, Strahilevitz J, Sahm D, Jacoby GA,Hooper DC (2006)*qnr*prevalence in ceftazidime-resistant *Enterobacteriaceae* isolates from the United States. Antimicrob Agents Chemother 50:2872-2874.

Ruzauskas M, Siugzdiniene R, Klimiene I, Virgailis M, Mockeliunas R, Vaskeviciute L, Zienius D (2014) Prevalence of methicillin-resistant *Staphylococcus haemolyticus* in companion animals: a cross-sectional study. Ann Clin Microbiol Antimicrob13:56.

Sandvang D, Aarestrupp FM (2009)Characterization of aminoglycoside resistance genes and Class 1 integrons in porcine and bovine gentamicin-resistant *Escherichia coli.*Microb Drug Resist6:19-27.

SeputieneV, Povilonis J, Ruzauskas M, Pavilonis A, Suziedeliene E (2010) Prevalence of trimethoprim resistance genes in *Escherichia coli* isolates of human and animal origin in Lithuania. J Med Microbiol59:315-322.

SwansonSJ, SniderC, BradenCR, BoxrudD, WunschmannA, RudroffJA, LockettJ,SmithKE (2007)Multidrug-resistant *Salmonella enterica* serotype *Typhimurium* associated with pet rodents. N Engl J Med356:21-28.

Thong KL, Bakeri SA, Lai KS, Koh YT, Taib MZ, Lim VKE, Yasin RM (2004) Molecular subtyping of Salmonella enterica serovar Tshiongwe recently isolated in Malaysia during 2001-2002. Southeast Asian J Trop Med Public Health 35:92-96.

Tietjen M, Fung DYC (1995)*Salmonellae* and food safety. Crit Rev Microbiol 21:53-83.

Tirado C, Schmidt K (2001) WHO surveillance programme for control of foodborne infections and intoxications: preliminary results and trends across greater Europe. World Health Organization. J Infect43:80-84.

Vassort-Bruneau C, Lesage-Descauses M, Martel JL, Lafont JP, Chaslus-Dancla E (1996) CAT III chloramphenicol resistance in *Pasteurella haemolytica* and *Pasteurella multocida* isolated from calves. J Antimicrob Chemoth38: 205-213.

Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S (2016) Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in *Escherichia coli,* Belgium. Euro Surveill.21:pii=30280.

Yan JJ, WuJJ, Ko WC,Tsai SH, ChuangCL, Wu HM, Lu YJ, Li JD (2004) Plasmid-mediated 16S rRNA methylases conferring high-level aminoglycoside resistance in *Escherichia coli* and *Klebsiella pneumoniae* isolates from two Taiwanese hospitals. J Antimicrob Chemother 54:1007-1012.

Zhang L, Fu Y, Xiong Z, Ma Y, Wei Y, Qu X, Zhang H, Zhang J, Liao M (2018)Highly prevalent multidrug-resistant *Salmonella* from chicken and pork meat at retail markets in Guangdong, China.FrontMicrobiol9:2104.