Slov Vet Res 2018: 55 (3): 161 - 70
DOI 10.26873/SVR-465-2017
UDC 579.62:579.842.1/.2:615.015.8:615.33:57.083

Original Research Article

Salmonella Typhimurium BETWEEN 2000 AND 2012: ANTIMICROBIAL RESISTANCE AND PFGE PATTERNS OF ISOLATES FROM ANIMALS, HUMANS AND FOOD

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Abstract: *Salmonella* Typhimurium is an important zoonotic pathogen with high levels of antimicrobial resistance. In the present study, we compared the pulsed-field gel electrophoresis (PFGE) and antimicrobial resistance patterns of 275 *S.* Typhimurium isolates collected between 2000 and 2012: 93 from humans, 111 from animals and 71 from food. A high rate of antimicrobial resistance was detected (71.6%). Multidrug resistance (MDR), defined as phenotypic resistance to three or more antimicrobial classes, was detected in more than half of the isolates (54.9%). The proportion of MDR isolates was the highest in animals (43%), followed by food (30.5%) and humans (26.5%). Among 27 phenotypically determined resistance patterns, three were found to be most common: ACNaSuT (19.3%), ACSuT (12%) and ASuT (11.3%). The first two patterns were the most prevalent in animal isolates (47.2% and 51.5%, respectively), while ASuT isolates were most commonly obtained from humans (58.1%). Macrorestriction with *Xba*I revealed 72 pulsotypes in nine clusters (A to I) and 19 unique pulsotypes (X1-X19). By far largest cluster F with 29 pulsotypes comprised 58.2% of tested isolates and included 53.8% animal, 26.3% food and 20% human isolates. Two thirds (66.3%) of the isolates in this cluster were MDR. The second largest was cluster E with 11.4% isolates of 12 pulsotypes, including 59.4% human, 31.3% food and 9.4% animal isolates. MDR was detected in 78.1% isolates with the most prevalent resistance pattern ASuT. Cluster I (16 isolates, 2 genotypes) consisted of 87.5% human and 12.5% animal isolates. The majority of these isolates (81.3%) were sensitive to tested antimicrobials and MDR isolates (12.5%) were of ASuT resistance pattern. This work provided valuable data about resistance and PFGE patterns of *S.* Typhimurium isolates in Slovenia and as global knowledge is essential for improved surveillance of the infections, the data obtained could serve as a base for both national and multistate outbreak investigations.

Key words: *Salmonella* Typhimurium; human; animal; food; PFGE; antimicrobial resistance

Introduction

Salmonella can colonize a variety of animals. As the microbe is excreted via faeces and can survive in the environment, the latter may serve as the source of human and animal infections. Salmonellosis is the second most frequent food-borne zoonosis in the European Union (EU) (1). The main sources of human infections are poultry meat, eggs and dairy products (2). One of the most important nontyphoidal *Salmonella* serovars, beside the most prevalent *Salmonella* Enteritidis, is *Salmonella* Typhimurium (*S.* Typhimurium), which was in 2015 detected in 15.8% humans, 56.9% pigs, 43.2% cows and 5.8% poultry in the EU (1). *S.* Typhimurium-related salmonellosis is a major public health problem, especially because of the microbe's high resistance to antimicrobials. Although human salmonellosis often occurs as a self-limiting disease with diarrhoea, abdominal cramps and fever, the risk groups (infants, elderly people and immunocompromised patients) may be endangered by life-threatening infections, for

Received: 12 September 2017
Accepted for publication: 28 November 2017

which effective antimicrobials are needed (3). Antimicrobial resistance varies among *Salmonella* serovars countries. In general, *S.* Typhimurium shows a higher level of resistance than *S.* Enteritidis. As reported on the EU level for 2015, only 38.5% *S.* Typhimurium isolates in humans were susceptible to all tested antimicrobials and 44.4% of isolates were multidrug resistant (MDR) (4). Resistance to ampicillin, sulphonamides and tetracyclines was frequently detected, while resistance to third-generation cephalosporins is rarely found in nontyphoidal *Salmonella* serovars. Meanwhile, resistance to clinically important fluoroquinolones varies between serovars from humans and animals in different countries. Among the EU Member States (MSs), Slovenia showed the highest proportion of human *S.* Typhimurium isolates resistant to ciprofloxacin (41.7%). Furthermore, monophasic variant of *S.* Typhimurium 4,[5],12:i:- is associated with an increased resistance with 81.1% MDR (4).

In Slovenia, *S.* Typhimurium was the fourth most common serovar isolated from humans (9.9%) in 2015 (5), while the prevalence of *S.* Typhimurium in pigs was 9%. In poultry, the prevalence in 2015 was below 1%, with only one positive broiler flock (6). The presence of *S.* Typhimurium in cattle is currently not monitored.

Molecular tools such as pulsed-field gel electrophoresis (PFGE) are indispensable for disease surveillance, detection of outbreaks and epidemiological investigations. PFGE is a standardized method based on restriction of genomic DNA and is currently considered the gold standard for subtyping of different bacterial foodborne pathogens.

The aim of our study was to characterize *S.* Typhimurium isolates from humans, animals and food collected within 12 years by means of susceptibility testing and PFGE and to compare the characteristics between the groups of isolates in order to elucidate their potential relationships.

Materials and methods

Bacterial isolates

S. Typhimurium isolates (n=275) from humans (n=93), animals (n=111) and food (n=71) in the period between 2000 and 2012 were included in this study (Table 1). Human isolates from clinical salmonellosis cases were obtained from four regional offices of the National Laboratory of Health, Environment and Food and the National Institute of Public Health (NIPH). Animal isolates from the Institute of Microbiology and Parasitology of the Veterinary Faculty (IMP-VF) collection originated from clinically ill animals and from routine *Salmonella* monitoring samples. Food isolates were from the IMP-VF and NIPH collections. Isolates were stored in stab tubes and inoculated into blood agar before typing. All isolates were additionally confirmed as *Salmonella* sp. with MALDI-TOF (Bruker, Germany).

Antimicrobial susceptibility testing

The antimicrobial susceptibility was performed by determining the minimum inhibitory concentration (MIC) by microdilution method using commercially available microplate (EUVSEC, Sensititre®, Trek Diagnostic Systems, Thermo Fisher Scientific, USA). All isolates were phenotypically tested for their susceptibility to 14 antimicrobials from nine different antimicrobial classes: ampicillin (A), cefotaxime (Fot), ceftazidime (Taz), meropenem (Mero), nalidixic acid (Na), ciprofloxacin (Cip), tetracycline (T), colistin (Col), gentamicin (G), trimethoprim (Tm), sulfamethoxazole (Su), chloramphenicol (C), azythromicin (Azi) and tigecycline (Tig). *Escherichia coli* ATCC 25922 was used as a test control strain. The results were interpreted according to the European Committee on Antibiotic Susceptibility Testing (EUCAST) epidemiological cut-offs (7) and the recommendations of the European Union Reference Laboratory for Antimicrobial Resistance (EURL AMR) (8). The interpretative criteria were in concordance with the Decision 2013/652/EU of the European Commission (4). Multidrug resistance (MDR) was defined as phenotypic resistance to three or more antimicrobial classes.

PFGE

PFGE was carried out according to the standardised PulseNet protocol (9). Restriction patterns were analysed with BioNumerics software (v. 6.6, Applied Maths, Belgium). The relation between two isolates was scored using the Dice coefficient of similarity. Cluster analysis was performed by the unweighted pair-group

method with arithmetic means (UPGMA). Position tolerance and optimisation were set at 1.5% and 1%, respectively. Bands of size less than 33.3 kb were excluded from the analysis. Isolates with PFGE profiles of >88% similarity were considered to belong to the same cluster (marked by letters). Within a cluster, profiles differing from each other in at least one band were considered as subtypes (marked by digits).

Results

Overall, less than one third (28.4%, 78/275) of isolates were susceptible to all tested antimicrobials. Susceptibility was more frequently encountered in isolates from humans than in isolates from animals and food (51.3% vs. 38.5% and 10.3% isolates, respectively). Among resistant isolates (71.6%, 197/275), penta-resistance was most frequently encountered (22.5%), followed by

Table 1: Background of *Salmonella* Typhimurium isolates studied

Isolate origin	Category	No. of isolates (n=275)	Year of isolation
humans	*Salmonella* Typhimurium-infected patients	93	2000-2012
animals	pigs	47	2000-2002, 2005-2010
	poultry	39	2000-2011
	cows	3	2005, 2007
	other (not used for human consumption)	22	2000-2001, 2004, 2006, 2008-2012
food	pork meat	19	2002, 2005-2009, 2011
	poultry meat	25	2001-2007, 2009-2011
	beef	2	2006, 2008
	rabbit	1	2005
	minced meat (pork+beef)	24	2002-2009, 2012

Table 2: Overview of susceptibility testing results of 275 *Salmonella* Typhimurium isolates from animals, humans and food

Resistance to No. of antimicrobial classes	Total	Humans	Animals	Food
	No. (%)	No. (%)	No. (%)	No. (%)
6	10 (3.6)	0 (0.0)	9 (8.1)	1 (1.4)
5	62 (22.5)	17 (18.3)	30 (27.0)	15 (21.1)
MDR ≥5	***72 (26.2)***	*17 (18.3)*	*39 (35,1)*	*16 (22.5)*
4	36 (13.1)	4 (4.3)	18 (16.2)	14 (19.7)
3	43 (15.6)	19 (20.4)	8 (7.2)	16 (22.5)
MDR	***151 (54.9)***	*40 (43.0)*	*65 (58.6)*	*46 (64.8)*
2	18 (6.5)	6 (6.5)	8 (7.2)	4 (5.6)
1	28 (10.2)	7 (7.5)	8 (7.2)	13 (18.3)
Susceptible	78 (28.4)	40 (43.0)	30 (27.0)	8 (11.3)
Total	275	93	111	71

Table 3: Resistance patterns and PFGE clusters of 275 *Salmonella* Typhimurim isolates from humans, animals and food

Resistance to No. of antimicrobial clases	Resistance pattern	Cluster	No. (%)	Humans No. (%)	Animals No. (%)	Food No. (%)
6	ACGNaSuT	F	**6 (2.2)**	-	6 (100.0)	-
6	ACGNaSuTTm	F, X[a]	**4 (1.5)**	-	3 (75.0)	1 (25.0)
5	ACGSuT	F, E	**2 (0.7)**	1 (50.0)	-	1 (50.0)
5	**ACNaSuT**	**F**	**53 (19.3)**	**15 (28.3)**	**25 (47.2)**	**13 (24.5)**
5	ACNaSuTTm	F	**7 (2.5)**	1 (14.3)	5 (71.4)	1 (14.3)
≥5	*MDR ≥5*	*E, F, X*	*72 (26.2)*	*17 (23.6)*	*39 (54.2)*	*16 (22.2)*
4	ACSuTTm	F	**1 (0.4)**	1 (100.0)	-	-
4	ANaSuT	F	**1 (0.4)**	-	-	1 (100.0)
4	CGNaSuTm	F	**1 (0.4)**	-	1 (100.0)	-
4	**ACSuT**	**F, G, X[a]**	**33 (12.0)**	**3 (9.1)**	**17 (51.5)**	**13 (39.4)**
3	ACSu	F	**1 (0.4)**	-	1 (100.0)	-
3	AGT	E	**1 (0.4)**	-	1 (100.0)	-
3	ANaSu	F	**1 (0.4)**	-	-	1 (100.0)
3	ANaSuTm	E	**1 (0.4)**	-	-	1 (100.0)
3	**ASuT**	**E, C, D, I, X[a]**	**31 (11.3)**	**18 (58.1)**	**3 (9.7)**	**10 (32.3)**
3	ASuTTm	A, X[a]	**4 (1.5)**	1 (25.0)	-	3 (75.0)
3	ATTm	B	**1 (0.4)**	-	-	1 (100.0)
3	GNaSu	F	**2 (0.7)**	-	2 (100.0)	-
3	GSuT	F	**1 (0.4)**	-	1 (100.0)	-
≥3	*MDR*	*A, B, C, D, E, F, G, I*	*151 (54.9)*	*40 (26.5)*	*65 (43.0)*	*46 (30.5)*
2	ASu	E, F, G, X[a]	**6 (2.2)**	3 (50.0)	1 (16.7)	2 (33.3)
2	AT	X[a]	**1 (0.4)**	-	-	1 (100.0)
2	GSu	F	**1 (0.4)**	-	1 (100.0)	-
2	NaSu	F	**7 (2.5)**	1 (14.3)	6 (85.7)	
2	SuT	H, G	**3 (1.1)**	2 (66.7)	-	1 (33.3)
1	G	G	**3 (1.1)**	2 (66.7)	1 (33.3)	-
1	Na	F, X[a]	**4 (1.5)**	-	1 (25.0)	3 (75.0)
1	Su	F, I	**15 (5.5)**	1 (6.7)	5 (33.3)	9 (60.0)
1	T	C, A, G, E, H	**6 (2.2)**	4 (66.7)	1 (16.7)	1 (16.7)
0	susceptible	F, G, I, H, E, B, X[a]	**78 (28.4)**	40 (51.3)	30 (38.5)	8 (10.3)
	Total		**275**	93 (33.8)	111 (40.4)	71 (25.8)

Table 4: Distribution of MICs and resistance of 275 *Salmonella* Typhimurium isolates from animals, humans and food, collected between 2000 and 2012

Antimicrobial		Resistance No.	MIC distribution (mg/l) - number and percent of isolates																		Range	MIC_{50}	MIC_{90}
		%	0,008	0.015	0.03	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024			
Ampicillin	A	154								70	48	3				1	153					1 - >64	>64	>64
		56,0								25,5	17,5	1,1				0,4	55,6							
Cefotaxime	Fot	0						266	9													0,25 - 0,5	>0,25	>0,25
		0,0						96,7	3,3															
Ceftazidime	Taz	0							271	4												0,5 - 1	>0,5	>0,5
		0,0							98,5	1,5														
Meropenem	Mero	0			259	16																0,03 - 0,064	>0,03	>0,03
		0,0			94,2	5,8																		
Nalidixic acid	Na	87										179	8	1		1	18	68				4 - >128	>4	>128
		31,6										65,1	2,9	0,4		0,4	6,5	24,7						
Ciprofloxsacin	Cip	87		151	37		39	44	3	1												0,015 - 1	>0,015	>0,25
		31,6		54,9	13,5		14,2	16,0	1,1	0,4														
Tetracycline	T	155									117	3		2	48	54	51					2 - >64	>32	>64
		56,4									42,5	17,6		0,7	17,5	19,6	18,5							
Colistin	Col	0								240	35											1 - 2	>1	>2
		0,0								87,3	12,7													
Gentamicin	G	21							240	12	2	2	2	2	5	10						0,5 - >32	>0,5	>1
		7,6							87,3	4,4	0,7	0,7	0,7	0,7	1,8	3,6								
Trimethoprim	Tm	19						234	18	4						19						0,25 - >32	>0,25	>0,5
		6,9						85,1	6,5	1,5					0,0	6,9								
Sulfamethoxazole	Su	181											21	23	36	11	3				181	8 - >1024	>1024	>1024
		65,8											7,6	8,4	13,1	4,0	1,1				65,8			
Chloramphenicol	C	108											166	1	1	3	39	65				8 - >128	>8	>128
		39,3											60,4	0,4	0,4	1,1	14,2	23,6						
Azithromycin	Azi	0,0									115	141	15	4								2 - 16	>4	>4
		0,0									41,8	51,3	5,5	1,5										
Tigecycline	Tig	0						168	97	10												0,25 - 1	>0,25	>0,5
		0,0						61,1	35,3	3,6														

resistance to three (15.6%), four (13.1%) and one (10.2%) class of antimicrobials. Resistance to two and six classes was less common, seen in 6.5% and 3.6% of isolates. More than half of all isolates (54.9%, 151/275) were determined as MDR. The proportion of all MDR isolates was the highest in animals (43%), followed by food (30.5%) and humans (26.5%). Among animal isolates, MDR was mostly found in isolates from farm animals (68.5% in farm animals vs. 18.2% in non-farm animals, data not shown). Detailed information about susceptibility testing results is shown in Table 2.

Among 27 phenotypically determined resistance patterns, three were found to be most common: ACNaSuT (19.3%, 53/275), ACSuT (12%, 33/275) and ASuT (11.3%, 31/275), while the remaining patterns were detected in lower proportions (range 0.4% to 5.5%). Resistance patterns ACNaSuT and ACSuT were the most prevalent in animal isolates (47.2% and 51.5%, respectively), while ASuT isolates were most commonly obtained from humans (58.1%).

Correlation between resistance patterns and genotypes was not evident. The majority of resistance patterns (i.e. 19) were related to only one PFGE cluster, while seven resistance patterns were found in two or more clusters. The most prevalent resistance pattern ACNaSuT was confined to cluster F, ACSuT isolates were found in clusters F and G and ASuT isolates in four clusters (E, C, D and I). The findings related to resistance patterns are summarized in Table 3, while MIC distribution is shown in Table 4.

Macrorestriction with *Xba*I revealed 72 pulsotypes in nine clusters (A to I) and 19 unique pulsotypes (X1-X19). Human isolates exhibited 31 distinct pulsotypes grouped in seven clusters and seven unique pulsotypes. In animal isolates, 34 different pulsotypes were distributed into seven clusters; in addition, nine unique pulsotypes were identified. Among food isolates, three non-clustered pulsotypes were detected, while 25 pulsotypes grouped in eight clusters. An overview of PFGE clusters and pulsotypes is given in Table 5.

By far the largest cluster F with 29 pulsotypes comprised 58.2% (160/275) of tested isolates and included 53.8% animal, 26.3% food and 20% human isolates. Two thirds (66.3%) of the isolates in this cluster were MDR. The most common pulsotype in this cluster was F9, encompassing 51.3% (82/160) of isolates in the cluster (data not shown). Isolates with F9 pulsotype were of animal (46.3%), food (34.1%) and human (19.5%) origin. About three quarters (76.8%) of F9 isolates were MDR. The two most common resistance patterns of F9 isolates were ACNaSuT and ACSuT. The former was detected only in cluster F and the latter was identified in a vast proportion (87.9%) in the same cluster.

The second largest was cluster E with 32/275 (11.4%) isolates of 12 pulsotypes, including 59.4% human, 31.3% food and 9.4% animal isolates. MDR was detected in 78.1% isolates with the most prevalent resistance pattern ASuT.

Cluster I (16 isolates, 2 genotypes) consisted of 87.5% human and 12.5% animal isolates.

Table 5: Distribution of PFGE clusters among 275 *Salmonella* Typhimurim isolates from humans, animals and food

Cluster	No. of PFGE patterns	Isolates No. (% of total)	Humans No. (% of cluster)	Animals No. (% of cluster)	Food No. (% of cluster)
A	4	4 (1.5)	1 (25.0)	0 (0.0)	3 (75.0)
B	4	4 (1.5)	0 (0.0)	1 (25.0)	3 (75.0)
C	3	6 (2.2)	4 (66.7)	1 16.7)	1 (16.7)
D	1	2 (0.7)	0 (0.0)	0 (0.0)	2 (100.0)
E	12	32 (11.6)	19 (59.4)	3 (9.4)	10 (31.3)
F	29	160 (58.2)	32 (20.0)	86 (53.8)	42 (26.3)
G	10	20 (7.3)	12 (60.0)	5 (25.0)	3 (15.0)
H	7	12 (4.4)	4 (33.3)	4 (33.3)	4 (33.3)
I	2	16 (5.8)	14 (87.5)	2 (12.5)	0 (0.0)
X[a]	19	19 (6.9)	7 (36.8)	9 (47.4)	3 (15.8)
Total	91	275	93	111	71

[a] unique PFGE patterns

The majority of isolates (81.3%) were sensitive to tested antimicrobials, MDR isolates (12.5%) were of ASuT resistance pattern.

The remaining clusters were smaller, comprising 2 to 20 isolates and 2 to 10 genotypes (Table 5).

Discussion

S. Typhimurium isolates investigated in the present study exhibited a high level of antimicrobial resistance as only 28.4% isolates were susceptible to all antimicrobials tested. *S.* Typhimurium and monophasic *S.* Typhimurium are reported to contribute significantly to the overall numbers of MDR *Salmonella* in Europe (4). More than half of *S.* Typhimurium isolates from humans in the EU were resistant to ampicillin (56.3%), sulfonamides (52.4%) and tetracycline (51.9%) with high to extremely high levels in most reporting MSs. Even higher proportions of resistance were observed in monophasic *S.* Typhimurium from humans, where close to 90% of all isolates were resistant to these three antimicrobials. The proportions of isolates resistant to either of the two clinically most critical antimicrobials were rare (on average 6.6% for ciprofloxacin and 1.1% for cefotaxime). This is in concordance with the findings of the present study even though it is challenging to compare the results of resistance to ampicillin, sulfonamides and tetracycline for human isolates due to low numbers of tested isolates per year (range 1 to 19). When more than 10 isolates were tested within the same year, the resistance levels ranged from 31.6% to 55.6% for sulfonamides, 26.3% to 50% for tetracycline and 26.3% to 55.6% for ampicillin (data not shown). Resistance to cefotaxime, ceftazidime, meropenem, colistin, azithromycin and tigecycline was not recorded. A total of ten monophasic *S.* Typhimurium isolates were tested; three from humans, two from animals and five from food (data not shown). All isolates with the exception of one from food were MDR. All isolates were resistant to ampicillin and sulfamethoxazole and nine of them also to tetracycline.

Slovenia was ranked fourth among the MSs with extremely high and very high *S.* Typhimurium MDR with 55.3% MDR human isolates in 2015 (4). In addition, the temporal trend analysis performed for the 3 years 2013–2015 showed statistically significant increases in (fluoro)quinolone resistance in Slovenia. Furthermore, the highest proportion of isolates resistant to ciprofloxacin was reported from Slovenia (41.7%), but the number of isolates tested was low (n = 48). Reduced susceptibility of human isolates to ciprofloxacin is of special concern in Italy where 91.7% of isolates had MIC ≥ 0.125 mg/l, which is the cut-off value according EUCAST (10). Resistance to ampicillin and tetracycline also increased significantly in Slovenia (4). As mentioned before, differences in number of tested isolates per year in the present study render relevant comparison impossible, but the overall (2000-2012) MDR in human isolates was found to be 43% (Table 2), with 18.3% isolates being resistant to five classes of antimicrobials. This is comparable to the data in the EU in 2015 when MDR was reported to be 44.4% in *S.* Typhimurium and 81.1% in monophasic *S.* Typhimurium. Half of the MSs testing isolates for the nine antimicrobial classes included in the MDR analysis reported a few isolates resistant to at least six of the classes (4).

The resistance levels of human and animal *S.* Typhimurium isolates are even more concerning in China as 84.6% of tested isolates were MDR with 22 isolates resistant also to cephalosporines and ciprofloxacin. Additionally, six isolates were also resistant to azithromycin, which is the drug of choice for invasive infections (11, 12). Another study reported about an isolate from retail market, which was resistant to ten antimicrobials. This may represent substantial risk to public health in the future due to food industry globalization and the fact that resistance genes can be easely disseminated between bacteria (13).

The number of MDR *S.* Typhimurium isolates from human varies between countries; from only 11% in Finland, 29% in USA, 43.9% in Belgium to 86.8% in China (14, 15, 16, 17). These studies reported ACSSuT resistance pattern as the most prevalent in humans which contrasts the results of the present study where only 9.1% of human isolates showed ACSuT pattern (susceptibility to streptomycin was not tested). The most common MDR pattern in human isolates found in the present study was ASuT (58.1%), followed by ACNaSuT (28.3%).

Antimicrobials such as ampicillin, sulfamethoxazole and tetracycline have been widely used for many years in veterinary medicine to treat infections in production animals. Generally, MSs reported high levels of resistance to these antimicrobials from producing animals and meat products thereof. Overall, very high resistance to

ampicillin, sulfamethoxazole and tetracycline was observed in *S.* Typhimurium isolates from carcases of fattening pigs in the EU; 52.4% isolates were MDR and the most frequent MDR core pattern was ASuT. According to the data from the present study, ASuT resistance pattern was the third most prevalent among the studied isolates, being shown by 11.3% isolates. However, 58.1% of ASuT isolates were of human origin in contrast to only 9.7% of animal origin. But this pattern, along with the additional resistance to chloramphenicol and nalidixic acid, was the most prevalent in isolates studied (19.3%), especially in animals (47.2%). In pigs, 76.6% isolates were MDR, 29.8% of them showing ACNaSuT resistance pattern. For comparison, in Belgium only 31.2% pig isolates showed MDR pattern and as little as 3.4% isolates were resistant to nalidixic acid, which was in concordance with MDR prevalence in pork (16). Even better situation was reported in Sweden, where only 11% of animal isolates were MDR with the most common resistance pattern ACSSuT (18). In poultry, a slightly lower proportion of MDR compared to pigs was detected in the present study (59%) with the most prevalent resistance pattern being ACSuT. Resistance to nalidixic acid, which is an indicator agent for fluoroquinolone resistance prediction, was detected in 33.3% poultry isolates in contrast to 70.2% in pig isolates (data not shown). A high prevalence of nalidixic acid resistance was expected for isolates from pork meat, but it was only deteceted in 31.6%. Data about the origin of the pork meat could perhaps clarify the findings, as lower prevalence of nalidixic acid resistance could linked to imported pork meat. On the contrary, the proportions of nalidixic acid resistant isolates from poultry meat (32%) and beef meat (50%) were comparable with the proportions detected in isolates from poultry (33.3%) and cattle (66.7%). Similarly, a difference between pig and pork meat isolates was observed for chloramphenicol resistance (63.8% vs. 31.6%), while the proportions were comparable for other tested antimicrobials (data not shown). A notable difference in the number of MDR isolates was observed between production and non-production animals, as only 18.2% non-production animals were MDR compared to 68.5% farm animals (data not shown). This suggests that the food animal production technology should probably implement measures to prevent the outbreaks of the diseases, to stop the spread of bacterial pathogens and apply consistent laboratory diagnostics, which could lead to the reduction of antibiotic use and empiric treatment of the diseases. Resistance to five or more antimicrobial classes was seen almost exclusively in isolates from production animals with the exception of one isolate from a mouse which was caught on a poultry farm. Isolate from the mouse shared PFGE (genotype F9) and resistance characteristics (ACNaSuT) with isolates from poultry in the same time period which may be either a coincidence or may indicate mice as vectors of *S.* Typhimurium on farms. Furthermore, the same resistance pattern and genotype as in mouse was also detected in poultry meat and in humans in 2011.

PFGE analysis provided an insight into genetic diversity of isolates; a total of 91 PFGE patterns among 275 isolates were defined. Reports on *S.* Typhimurium diversity vary; Rounds et al. (19) described the serovar to be of low clonality in congruence with a few studies, while in several other reports the isolates exhibited high genetic similarity (20). Nineteen out of 91 patterns in the present study were unique and 40.7% of isolates grouped into three clusters (E, F and G). For comparison, *S.* Infantis isolates in Slovenia were found to be more genetically homogeneous as 74.7% of isolates grouped in only two clusters (data not published). PFGE has been used for epidemiological investigations to study *S.* Typhimurium PFGE patterns in the food chain and it has been demonstrated that genetically similar isolates were transferred along the slaughterhouse line and to retail markets (13). Identical PFGE patterns (e.g. F1, F7, F9) from animals, food and humans have been identified also in the present study and five of a total of nine *S.* Typhimurium clusters included isolates of all three origins. However, unfortunately there were no evident epidemiological links revealed as the isolates analysed were geographically and temporally scattered. Interestingly, the largest cluster F contained 58.2% of analysed isolates and 51.3% among them were characterized by F9 pattern which seems to be widespread, perhaps due to its temporal and genetic stability as described before (17). By comprising 77.5% of all animal isolates in the study, cluster F was mainly linked to animal isolates. Overall, 66.3% isolates in this cluster were MDR with common resistance patterns ACSuT and ACNaSuT, which were found to be related mostly to poultry and

pigs, respectively. On the other hand, clusters E, G and I comprised mostly human isolates; overall MDR in these clusters was 41.2% and common resistance pattern was ASuT.

Discussion about the detected PFGE patterns among different studies is hampered by the fact that reliable comparisons can only be achieved by using common PFGE databases. Ongoing establishment of a joint database for foodborne pathogens on the EU level, following the example of PulseNet, will undoubtedly facilitate interlaboratory comparison of typing results and enhance the surveillance of foodborne pathogens in the food chain.

In conclusion, the number of *S.* Typhimurium isolates in humans and animals does not seem to change much over the years; however, because of the worrying resistance levels, monitoring of all categories of production animals should be implemented in every MS. Lienemann et al. (14) suggested that the source of MDR *S.* Typhimurium for humans in Finland could be imported food sold in supermarkets and restaurants as MDR *Salmonella* is rare among domestic animal production. Therefore, global knowledge about prevalence, resistance patterns and genetic characteristics is essential for successful control of outbreaks on the international level. This work, the first of its kind in Slovenia regarding *S.* Typhimurium, provided valuable data about resistance and PFGE patterns of *S.* Typhimurium isolates from animals, humans and food on the national level and the data obtained could serve as a base for both national and multistate outbreak investigations.

Acknowledgments

The authors acknowledge the financial support from the Slovenian Research Agency (research core funding No. P4-0092). Špela Baus, Darja Kušar and Maja Kavalič are thanked for technical assistance and Vojka Bole-Hribovšek for critical reading of the manuscript.

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Salmonella Typhimurium MED LETOMA 2000 IN 2012: VZORCI ODPORNOSTI PROTI PROTIMIKROBNIM ZDRAVILOM IN VZORCI PFGE IZOLATOV IZ ŽIVALI, LJUDI IN ŽIVIL

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Povzetek: *Salmonella* Typhimurium *je* pomembna povzročiteljica zoonoz, pri katerih se pojavlja visok odstotek proti protimikrobnim zdravilom odpornih sevov. V raziskavi smo primerjali restrikcijske vzorce, pridobljene z metodo elektroforeze v pulzirajočem električnem polju (PFGE), in vzorce odpornosti 275 izolatov *S.* Typhimurium, izoliranih med letoma 2000 in 2012 iz 93 ljudi, 111 živali in 71 vzorcev hrane. Dokazali smo visok odstotek odpornih sevov (71,6 %). Odpornost proti trem ali več skupinam protimikrobnih zdravil (VOB) smo ugotovili pri več kot polovici izolatov (54,9 %). Največ VOB izolatov smo ugotovili pri živalih (43 %), sledita hrana (30,5 %) in ljudje (26,5 %). Med 27 fenotipskimi vzorci odpornosti so bili najpogostejši trije: ACNaSuT (19,3 %), ACSuT (12 %) in ASuT (11,3 %). Prva dva vzorca sta bila najpogostejša pri živalih (47,2 % in 51,5 %), medtem ko so bili izolati z vzorcem ASuT najpogosteje dokazani pri ljudeh (58,1 %). Na podlagi makrorestrikcije z encimom *Xba*I smo ugotovili 72 vzorcev PFGE, razvrščenih v devet genetskih skupin (A – I), in 19 edinstvenih vzorcev (X1 – X19). Največja je bila genetska skupina F z 29 vzorci, ki so predstavljali 58,2 % vseh izolatov (53,8 % iz živali, 26,3 % iz hrane in 20 % iz ljudi). Dve tretjini (66,3 %) izolatov v genetski skupini sta bili VOB. Druga največja je bila genetska skupina E z 11,4 % vseh izolatov (12 vzorcev PFGE), od tega 59,4 % iz ljudi, 31,3 % iz hrane in 9,4 % iz živali. VOB je bila dokazana pri 78,1 % izolatov z najpogostejšim vzorcem odpornosti ASuT. V genetski skupini I (16 izolatov, 2 genotipa) je bilo 87,5 % izolatov iz ljudi in 12,5 % izolatov iz živali. Večina teh izolatov (81,3 %) je bila občutljiva na vsa testirana protimikrobna zdravila, medtem ko so izolati VOB (12,5 %) imeli vzorec ASuT. V raziskavi smo pridobili dragocene podatke o odpornosti in vzorcih PFGE bakterije *S.* Typhimurium na nacionalni ravni. Za uspešen nadzor izbruhov je zelo pomembno globalno poznavanje te tematike, zato so podatki iz te raziskave pomemben prispevek k nacionalnemu in mednarodnemu preiskovanju izbruhov.

Ključne besede: *Salmonella* Typhimurium; človek; žival; hrana; PFGE; odpornost