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Some pathogenic characters of paratyphoid *Salmonella enterica* strains isolated from poultry

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ABSTRACT

Objective: To investigate some pathogenic characters of *Salmonella enterica* strains isolated from poultry.

Methods: Twenty-three genetically distinct *Salmonella enterica* strains, of different serovars and pulsotype, were examined for virulence traits. Resistance to gastric acid environment was estimated by measuring the percentage of survived bacterial cells after exposure for 2 h to a synthetic gastric juice. Strains were analyzed with PCR for the presence of the following virulence genes: *mgtC* and *rhuM* located on SPI-3, *sopB* and *pipB* located on SPI-5, *Salmonella* virulence plasmid (*spv*) R (*spvR*), *spvB* and *spvC* located on *Salmonella* plasmid virulence and *sodCI*, *sopE*, and *gipA* located on prophage. Finally, resistance to 21 antibiotics was tested with Kirby–Bauer method.

Results: A percentage of 82.60% of strains were resistant to gastric environment after induction and 60.87% of the strains exhibited constitutive resistance too. Nineteen different virulence profiles were detected. The phage related genes *sodCI* and *sopE* and the plasmid mediated operon *spvR, spvB and spvC* (*spvRBC*) were detected in 82.60%, 47.82% and 52.17% of strains, respectively. Typhimurium and Enteritidis strains showed the highest number of virulence genes. Twenty-one different antibiotic resistance profiles were obtained and two isolates (Typhimurium and Enteritidis) resulted sensible to all the tested molecules. The ampicillin, streptomycin, sulfonamide and tetracycline resistance profile was detected in seven isolates (30.43%).

Conclusion: Our results show that paratyphoid *Salmonella* strains with several characters of pathogenicity, that may be cause of severe pathology in animals and humans, are circulating among poultry.

1. Introduction

Salmonellosis is one of the most important zoonosis worldwide. The preferential route of transmission from animals to humans is through contaminated food or foodstuffs and, in particular, eggs, egg products and poultry meat are the primary source of infection for humans [1].

More than 2600 *Salmonella* serovars exist and all may be pathogenic for humans and animals, at least as cause of intestinal disorders [2]. However, only a limited number of serovars, mainly Typhimurium and Enteritidis, are most frequently associated to human infections [1].

Almost all salmonellae have salmonella pathogenicity island-1 (SPI-1) and salmonella pathogenicity island-2 (SPI-2), that include genes encoding for factors for intestinal and systemic infections, respectively [2,3].

However, more genes, less conserved in the genus *Salmonella*, determine the pathogenicity of this bacterium [4,5]. Salmonella pathogenicity island-3 (SPI-3) is involved in intracellular proliferation and Mg^{2+} uptake, and it contributes to systemic dissemination. Salmonella pathogenicity island-5 (SPI-5) has genes encoding for effector proteins for SPI-1 and SPI-2 and they are important to the development of intestinal symptoms and for intracellular surviving [4].

Virulence genes could be transferred between salmonellae by bacteriophage. In particular, many virulence factors carried on

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prophages have been described, for serovars Typhimurium and Enteritidis, like sopE and gipA involved in intestinal colonization, and sodCI, an enzyme that protects salmonellae from the "oxidative burst" in macrophage environment [6,7]. Moreover, many non-typhoid *Salmonella* strains associated with extra-intestinal infections in humans and animals carry an additional locus termed *Salmonella* virulence plasmid (*spv*), located on *Salmonella* virulence plasmid [8].

Resistance to antimicrobials influences the infection development. This could be related to different factors: treatment failure and consequent persistence of the infection, disruption of the normal competitive gut flora and, moreover, it is demonstrated that antibiotics can directly improve the bacterial virulence [9].

The aim of the present study was to investigate some pathogenic characters of *Salmonella enterica* (*S. enterica*) strains isolated from poultry, in particular: 1) resistance to gastric acid environment, 2) presence of virulence genes of SPI-3, SPI-5, plasmids and prophages, 3) antibiotic resistance.

2. Material and methods

2.1. Bacterial strains

Forty-four *S. enterica* strains isolated from 2010 to 2014 were selected for the study. All strains were isolated from asymptomatic poultry during routine investigations. The isolates included: 19 *S.* ser. Enteritidis, 13 *S.* ser. Typhimurium, 8 *S.* ser. Infantis, 3 *S.* ser. Typhimurium monophasic variant and 1 *S.* ser. Thompson.

Isolates were screened with pulsed field gel electrophoresis, following the protocol reported by other authors [10], and only the strains belonging to different pulsotypes were further analyzed in order to avoid isolates redundancy.

2.2. Resistance to gastric environment

Constitutive and inducible gastric acid resistance was evaluated following the protocol previously described by Xia *et al* [11]. Briefly, *S. enterica* isolates were grown at 37 °C overnight in LB-MOPS (Luria Bertani broth, plus morpholinepropanesulfonic acid, 100 mmol/L, pH 8.0) and LB-MES (Luria Bertani broth, plus morpholineethanesulfonic acid, 100 mmol/L, pH 5.5) broths to evaluate the constitutive and inducible resistance, respectively. Cultures were diluted 1:200 in synthetic gastric juice (8.3 g proteose–peptone, 3.5 g glucose, 2.05 g NaCl, 0.6 g KH_2PO_4, 0.11 g $CaCl_2$, 0.37 g KCl, 0.05 g porcine bile, 0.1 g lysozyme, 13.3 mg, ultrapure water 1 L; pH was adjusted to 3.0 with 6 mol/L HCl) and incubated at 37 °C in water bath for 2 h. Viable cell counts were determined before and after incubation by plating serial dilutions in PBS (pH 7.2) on LB agar. Results were expressed as the percentage of survived cells after synthetic gastric juice challenge. Three replicates were done for each strain. The minimum percentage of survived cells to consider a strain resistant was fixed to 1%.

2.3. Presence of virulence genes

DNA was extracted from overnight cultures of each isolate using the DNeasy Blood and Tissue Kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions and stored at 4 °C until used as template for PCR assays.

The presence of *spvR*, *spvB*, *spvC*, *sodCI*, *mgtC*, *sopE*, *sopB*, *pipB*, *rhuM* and *gipA* genes was evaluated. Table 1 shows target genes, their location and amplification products size. Single PCR was executed for each gene, following protocols reported by other authors [7,12–15].

2.4. Antibiotic resistance

Resistance to 22 antibiotics was evaluated by the standard disk diffusion method of Kirby–Bauer, on Mueller Hinton Agar (Oxoid, Basingstoke, UK), as describe in Clinical and Laboratory Standards Institute (CLSI) manual [16]. The following antibiotics were employed (Oxoid): amoxycillin–clavulanic acid (30 μg), ampicillin (10 μg), amikacin (30 μg), cephalothin (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), colistin (10 μg), enrofloxacin (5 μg), florfenicol (30 μg), gentamycin (10 μg), kanamycin (30 μg), nalidixic acid (2 μg), nitrofurantoin (300 μg), streptomycin (10 μg), trimethoprim-sulfamethoxazole (25 μg), sulfonamide (300 μg), tetracycline (30 μg), tigecycline (15 μg), tobramycin (10 μg), trimethoprim (5 μg).

Results were interpreted following European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables and, where not possible, according to National Committee for Clinical Laboratory Standards (NCCLS) indications [17,18].

3. Results

3.1. Bacterial strains

Twenty-three *Salmonella* strains were selected on the basis of the pulsed field gel electrophoresis results. Ten different pulsotypes were obtained for ser. Typhimurium, 9 for ser. Enteritidis, 2 for ser. Typhimurium monophasic variant, and 1 for ser. Infantis.

3.2. Resistance to gastric environment

Four strains (17.39%), one *S.* ser. Thompson and three *S.* ser. Enteritis, resulted sensible to the gastric acid environment, both before and after induction. Five strains (21.74%) were resistant only after induction. The remaining 14 strains (60.87%) showed both constitutive and induced resistance. Table 2 reports the results obtained for each analyzed strain, in relation with virulence genes and antibiotic resistance profiles.

3.3. Presence of virulence genes

The genes *spvR*, *spvB* and *spvC* (*spvRBC*) were always found in association. The gene *sodCI* was detected in 19/23 (82.60%) strains, *mgtC* in 13/23 (56.52%), *spvRBC* in 12/23 (52.17%) strains, *sopE* in 11, *sopB* in 11 and *pipB* in 11/23 (47.82%) strains, *rhuM* in 4/23 (17.39%) strains and *gipA* in 2/23 (8.69%) strains.

Table 3 shows the distribution of the virulence genes among the analyzed serovars. In particular, the *S.* ser. Infantis isolate did not show any investigated genes. *S.* ser. Thompson strains had only *sopB* and *pipB* genes. The genes *sopE* and *spvRBC* were detected in Enteritis and Typhimurium strains. *gipA* was observed only in Typhimurium isolates. A total of 19 different profiles were detected.

Table 1
Investigated virulence genes, employed primers and expected PCR product size.

Gene	Location	Primers	Sequence (5′→3′)	Product size (bp)	Reference
mgtC	SPI3	mgtCF	CAT CGG CTG TAC CCG ACT AT	196	[12]
		mgtCR	CAG CAC GCT GAT GAA TGA GT		
pipB	SPI5	pipBF	AAT ATC GGA TGG GGG AAA AG	230	[12]
		pipBR	AAC CTG ACT CAC GCA GAC CT		
rhuM	SPI3	rhuMF	CAT CGG CTG TAC CCG ACT AT	222	[12]
		rhuMR	CAG CAC GCT GAT GAA TGA GT		
sopB	SPI5	sopBF	TCA GAA GRC GTC TAA CCA CTC	517	[13]
		sopBR	TAC CGT CCT CAT GCA CAC TC		
spvR	Plasmid	spvRF	GCA GTG CGT GAT CTG TTG AT	202	[14]
		spvRR	TTT CAT GAG GGG GCT AAA AA		
spvC	Plasmid	spvCF	AAT TTG CCG GTG ACA AGT TC	235	[14]
		spvCR	CGT GTC TTG TGG AGA AAC GA		
spvB	Plasmid	spvBF	CTA TCA GCC CCG CAC GGA GAG CAG TTT TTA	717	[15]
		spvBR	GGA GGA GGC GGT GGC GGT GGC ATC ATA		
gipA	Prophage	gipAF	ACG ACT GAG CAG GCT GAG	421	[13]
		gipAR	TTG GAA ATG GTG ACG GTA GAC		
sodCI	Prophage	sodCIF	TAT CGG AGT AAT TGT CAC CG	465	[7]
		sodCIR	ACA ATA TTG TCG CTG GTA GC		
sopE	Prophage	sopEF	AAT TCA TCA ATC AGA TGG AC	869	[7]
		sopER	TCA TAT TAA TCA GGA AGA GG		

Table 2
Gastric acid resistance, virulence genes and antibiotic resistance profiles of each analyzed *S. enterica* isolate.

Strain	Serovar	Gastric environment resistance (mean ± SD)		Virulence genes profile	Antibiotic resistance profile
		Constitutive	Induced		
S28	Typhimurium	1.08 ± 0.82	8.17 ± 0.47	*sopB rhuM pipB mgtC gipA*	S3
S111	Thompson	0.05 ± 0.06	0.31 ± 0.40	*sopB pipB*	AMP S3
S176	Typhimurium	2.81 ± 0.61	18.09 ± 3.64	*sodCI spvRBC sopB pipB mgtC gipA*	
S207	Infantis	3.77 ± 1.61	67.02 ± 7.94	no genes	NA S TE TGC S3 W SXT F
S217	Enteritidis	3.35 ± 1.99	74.50 ± 6.99	*sodCI sopE spvRBC*	NA TGC S3 F
S218	Enteritidis	4.23 ± 5.90	44.63 ± 7.74	*sodCI sopE*	NA TGC S3 F
S219	Enteritidis	0.12 ± 0.16	29.80 ± 10.61	*sodCI sopE spvRBC*	AMP AMC F
S220	Enteritidis	0.13 ± 0.16	29.38 ± 9.51	*sodCI sopE spvRBC*	S3
S221	Enteritidis	0.01 ± 0.01	0.01 ± 0.00	*sodCI sopE spvRBC*	S3 F
S232	Enteritidis	0.03 ± 0.04	0.39 ± 0.41	*sodCI sopE spvRBC sopB pipB*	S3 F
S233	Enteritidis	0.04 ± 0.05	0.01 ± 0.01	*sodCI sopE sopB rhuM mgtC*	S3 F
S234	Enteritidis	0.83 ± 1.17	3.11 ± 4.16	*sodCI sopE spvRBC sopB rhuM mgtC*	
S236	Enteritidis	21.61 ± 4.53	25.79 ± 7.45	*sodCI sopE spvRBC sopB*	S3 F
S239	Typhimurium monophasic variant	3.18 ± 3.59	40.27 ± 8.75	*sodCI rhuM*	AMP KF S TE S3 F
S240	Typhimurium	4.63 ± 3.54	35.91 ± 14.28	*sodCI sopB pipB mgtC*	AMP S AK TE TGC S3
S241	Typhimurium	14.78 ± 0.36	40.90 ± 0.75	*sodCI sopB pipB mgtC*	AMP KF S TE TGC S3
S244	Typhimurium monophasic variant	5.39 ± 5.10	41.04 ± 12.75	*sodCI mgtC*	AMP S TE TGC S3
S245	Typhimurium	9.62 ± 0.33	47.05 ± 10.97	*sodCI spvRBC sopB pipB mgtC*	TGC
S250	Typhimurium	4.17 ± 0.57	24.39 ± 3.52	*sodCI spvRBC sopB pipB mgtC*	S TGC
S251	Typhimurium	2.41 ± 3.32	16.57 ± 0.25	*sodCI sopE mgtC*	AMP S TE TGC S3
S252	Typhimurium	3.37 ± 2.33	16.88 ± 4.19	*sodCI sopE spvRBC pipB mgtC*	AMP S TE S3 F C FFC
S258	Typhimurium	0.59 ± 0.78	34.49 ± 1.64	*sodCI spvRBC pipB mgtC*	AMP AMC S TE TGC S3 F C FFC
S261	Typhimurium	0.12 ± 0.13	7.68 ± 5.64	*pipB mgtC*	S S3 F

AMC = amoxycillin-clavulanic acid, AMP = ampicillin, AK = amikacin, KF = cephalothin, C = chloramphenicol, FFC = florphenicol, F = nitrofurantoin, S = streptomycin, S3 = sulfonamide, TE = tetracycline, TGC = tigecycline.

3.4. Antibiotic resistance

All the 23 strains resulted sensible to ciprofloxacin and ceftazidime. Many strains (percentage $\geq$ 80%) were susceptible to nalidixic acid, enrofloxacin, cephotaxime, trimethoprim, trimethoprim-sulfamethoxazole, chloramphenicol and florfenicol. Resistance was mainly detected to ampicillin, streptomycin, tetracycline, tigecycline, sulfonamide and nitrofurantoin (Table 4).

Twenty-one different antibiotic resistance profiles were obtained. Only 2 isolates (8.69%) resulted sensible to all tested molecules. The remaining 21 strains (91.30%) were resistant to 1–9 antibiotics; in particular 10 strains (43.47%) resulted resistant to four or more classes of antibiotics.

Table 3
Distribution of virulence genes among different analyzed *Salmonella* isolates.

Serovar	Number of analyzed strains	Virulence genes							
		sodCI	*sopE*	*spvRBC*	*sopB*	*rhuM*	*pipB*	*mgtC*	*gipA*
Typhimurium	10	8	2	5	6	1	9	10	2
Enteritidis	9	9	9	7	4	2	1	2	0
Typhimurium monophasic variant	2	2	0	0	0	1	0	1	0
Infantis	1	0	0	0	0	0	0	0	0
Thompson	1	0	0	0	1	0	1	0	0
Total	23	19	11	12	11	4	11	13	2

Table 4
Antimicrobial resistance of *S. enterica* isolates [*n* (%)].

Antibiotic		Susceptible	Intermediate	Resistant	Non-susceptible
Fluoroquinolones	Nalidixic acid	20 (86.96)	0 (0.00)	3 (13.04)	3 (13.04)
	Ciprofloxacin	23 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
	Enrofloxacin	20 (86.96)	3 (13.04)	0 (0.00)	3 (13.04)
Penicillins	Ampicillin	14 (60.87)	0 (0.00)	9 (39.13)	9 (39.13)
	Amoxycillin-clavulanic acid	18 (78.26)	3 (13.04)	2 (8.70)	5 (21.74)
Cephems (cephalosporins)	Cephotaxime	20 (86.96)	3 (13.04)	0 (0.00)	3 (13.04)
	Cephalothin	18 (78.26)	3 (13.04)	2 (8.70)	5 (21.74)
	Cephtazidime	23 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
Aminoglycosides	Gentamycin	18 (78.26)	5 (21.74)	0 (0.00)	5 (21.74)
	Kanamycin	7 (30.43)	16 (69.57)	0 (0.00)	16 (69.57)
	Streptomycin	4 (17.39)	9 (39.13)	10 (43.48)	19 (82.61)
	Amikacin	15 (65.22)	7 (30.43)	1 (4.35)	8 (34.78)
	Tobramycin	14 (60.87)	9 (39.13)	0 (0.00)	9 (39.13)
Tetracyclines	Tetracycline	11 (47.83)	4 (17.39)	8 (34.78)	12 (52.17)
	Tigecycline	1 (4.35)	12 (52.17)	10 (43.48)	22 (95.65)
Folate pathway inhibitors	Sulfonamide	5 (21.74)	0 (0.00)	18 (78.26)	18 (78.26)
	Trimethoprim	22 (95.65)	0 (0.00)	1 (4.35)	1 (4.35)
	Trimethoprim-sulfamethoxazole	22 (95.65)	0 (0.00)	1 (4.35)	1 (4.35)
Others	Colistin	23 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)
	Nitrofurantoin	5 (21.74)	6 (26.09)	12 (52.17)	18 (78.26)
	Chloramphenicol	21 (91.30)	0 (0.00)	2 (8.70)	2 (8.70)
	Florphenicol	19 (82.61)	2 (8.70)	2 (8.70)	4 (17.39)

The resistance profile ampicillin, streptomycin, sulfonamide and tetracycline (ASSuT) was detected in 7 isolates (30.43%).

4. Discussion

The first character of pathogenicity evaluated in the present study was the resistance to gastric acid environment, because the ability to survive the gastric pH is essential for enteric pathogens to reach the intestine, primary site of infection. In particular, constitutive and inducible acid resistance was evaluated. The 60.87% of the strains exhibited both kinds of resistance, while the 21.74% of strains only showed inducible resistance. The ability to acquire resistance to low pH after growing in mildly acid condition represents an advantage for the foodborne pathogens. Our results showed that the majority of the tested strains had this property. Moreover, the isolates with constitutive resistance (Enteritidis, Infantis, and Typhimurium) after induction showed higher resistance, with percentages of survived cells until 74.5%.

The second investigated character was the presence of some virulence genes, present in SPI-3, SPI-5, prophages and plasmids. The most frequently detected gene was *sodCI*. This gene, carried by a phage, contributes to the survival of salmonellae in macrophage, protecting the bacteria from the "oxidative burst", and it is important for systemic dissemination [7,19]. All *S.* ser. Enteritidis, *S.* ser. Typhimurium monophasic variant, and 8/10 *S.* ser. Typhimurium isolates, included in this study, had this gene.

The *spv* was detected in 7/9 *S.* ser. Enteritidis and in 5/10 *S.* ser. Typhimurium, whereas none of the other isolates carried this gene. The s*pv* gene concurs to survival of salmonellae inside the macrophage and to their systemic spreading.

Eleven of 23 strains (47.82%) harbored the gene *sopE*. This gene, carried by a phage, is involved in epithelial cells invasion at intestinal level and stimulation of the host inflammatory response. The present study found *sopE* in all examined *S.* ser. Enteritidis and 2/10 Typhimurium strains.

The present survey found the gene *gipA* in two Typhimurium strains. It is involved in Peyer's patch survival and it concurs to salmonella colonization of the M cells in the ileal Peyer's patch [20].

The 47.82% of the strains had the genes *sopB* and *pipB*, both located on SPI-5. *sopB* contributes to enterocytes invasion and mediate fluid secretion, whereas *pipB* is involved in the bacteria survival in the intracellular environment [21,22].

Others investigated chromosomal genes were *mgtC* and *rhuM*, both located on SPI-3 and linked to the capacity to produce a systemic infection [23,24]. *mgtC*, important for the bacterial metabolism in the phagosome, resulted the second most frequently detected gene, with 56.52% of positive strains. On the other hand, only 4 strains (17.39%) harbored *rhuM*, all but one in association with *mgtC*.

The investigated genes, with the exception of *rhuM*, are considered by some authors to be present in most *Salmonella* isolates [25]. Our study found a limited spreading of these genes, as documented by other authors [13,14,25].

Nineteen different profiles were identified. Only one strain, ser. Infantis, resulted negative for all the investigated genes. The remaining strains had 2–6 genes in association and Typhimurium and Enteritidis strains resulted the most virulent serovars.

The last investigated feature was the antimicrobial resistance. Quinolones, cephalosporins, phenicols, amoxicillin–clavulanate, colistin, trimethoprim and the association trimethoprim–sulfamethoxazole resulted effective against more than 80% of tested strains.

Most of the isolates (78.26%) showed resistance to sulfonamide. This is one of the most common antimicrobials used in chicken flocks in a large number of countries, thus the observed high resistance is not surprising.

A total of 78.26% (52.17% resistant and 26.09% intermediate) resulted non-susceptible to nitrofurantoin. This is a molecule active against a broad range of bacteria, that in the past has been largely used in veterinary medicine. In 1993, in European Union (EU), it was banned because of the risk to human health due to the occurrence of toxic residues in food products. The currently detected resistance to nitrofurantoin could be related to a selection of resistant strains in the past or to its continuous illegal use [26].

A very high percentage (95.65%) of isolates resulted no-susceptible to tigecycline. This is a member of the glycylcycline group of antibiotics, and was registered in the EU in April 2006. It is a bacteriostatic antibiotic active against a broad range of bacteria, with only few naturally resistant exceptions. Specifically, tigecycline is considered effective against multidrug resistant bacteria, including Enterobacteriaceae. Reports of resistance to tigecycline have been rare in naturally susceptible pathogens; however, resistant variants may be encountered [27].

Our results suggest that resistance to tigecycline is not so rare and it represents an emerging problem for the bacterial treatment in veterinary and human medicine, as supposed by other authors [28].

Moderate resistance was observed against tetracycline, confirming that this antibiotic, largely employed in veterinary medicine, often is not effective in the treatment of bacterial infections, as reported by other studies [29,30].

The 82.61% (43.48% resistant and 39.13% intermediate) of isolates were no-susceptible to streptomycin. The tested isolates, even though not resistant, resulted intermediate to the other aminoglycosides.

Overall 16 different resistance profiles were observed. All but two strains resulted resistant at least to one antibiotic. In particular, 10 strains were resistant to four or more different antibiotics belonging to four or more different antibiotic classes. ASSuT profile was encountered in 5 *S.* ser Typhimurium and 2 *S.* ser Typhimurium monophasic variant isolates. This resistotype, usually found in Typhimurium and in its monophasic variant and often associated to resistance to other antibiotics, is considered typical of the most virulent *Salmonella* strains [31].

The present study shows that paratyphoid *Salmonella* strains with several characters of pathogenicity are circulating among poultry. Most of the isolates resulted able to survive to the gastric acid environment, especially after induction, a feature indispensable to establish intestine infection. Great variability was observed in the distribution of virulence genes; however, most of the investigated virulence genes were frequently detected in Enteritidis and Typhimurium isolates, confirming these serovars as the most potentially pathogenic salmonellae.

The antibiotic resistance is an increasing threat for the therapy of *Salmonella* and other bacterial infections. The present investigation found good effectiveness of fluoroquinolones, cephalosporins and colistin against the tested isolates. Surprising an high percentages of no-susceptible isolates were observed against tigecycline, a relatively new molecule often indicated for the *Salmonella* infection treatment, and nitrofurantoin that has been banned for several years.

No correlation was observed between antibiotic resistance and serovars, but ASSuT profile, considered as often associated to the most virulent strains, was detected only in serovars Typhimurium and Typhimurium monophasic variant.

The obtained results underline that poultry may be infected by virulent paratyphoid *Salmonella* strains. Even though infected animals often do not develop clinical disease, they represent a potential source of salmonellae for humans, because of the contamination of poultry products.

Conflict of interest statement

The authors declare no conflict of interest.

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