

Distribution of integrons and SGI1 among antibiotic resistant isolates of animal origin

H. Havlickova, H. Hradecka, I. Bernardyova, I. Rychlik

▶ To cite this version:

H. Havlickova, H. Hradecka, I. Bernardyova, I. Rychlik. Distribution of integrons and SGI1 among antibiotic resistant isolates of animal origin. Veterinary Microbiology, 2008, 133 (1-2), pp.193. 10.1016/j.vetmic.2008.06.027 . hal-00532450

HAL Id: hal-00532450 https://hal.science/hal-00532450

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Distribution of integrons and SGI1 among antibiotic resistant *Salmonella enterica* isolates of animal origin

Authors: H. Havlickova, H. Hradecka, I. Bernardyova, I. Rychlik

PII:	S0378-1135(08)00252-6
DOI:	doi:10.1016/j.vetmic.2008.06.027
Reference:	VETMIC 4083
To appear in:	VETMIC
Received date:	10-4-2008
Revised date:	13-6-2008
Accepted date:	26-6-2008



This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



1	Distribution of integrons and SGI1 among antibiotic resistant Salmonella enterica isolates of
2	animal origin
3	
4	
5	Havlickova H. ¹ , Hradecka H. ¹ , Bernardyova I. ² , Rychlik I. ¹ *
6	
7	
8	
9	¹ Veterinary Research Institute, Hudcova 70, 621 00 Brno, Czech Republic
10	
11	² State Veterinary Institute, Sidlistni 136, 165 03 Prague, Czech Republic
12	
13	
14	
15	
16	* Corresponding author
17	Ivan Rychlik
18	Veterinary Research Institute
19	Hudcova 70
20	621 00 Brno
21	Czech Republic
22	
23	phone +420 533331201
24	fax +420 541211229
25	Email: rychlik@vri.cz

26 Abstract

27 In Salmonella enterica, resistance to antibiotics can be caused by the presence of SGI1, 28 transposons or conjugative plasmids. In this study we were interested in the relative 29 contribution of these genetic elements to the antibiotic resistance of *S. enterica* isolates 30 collected within a single year in the Czech Republic from animal sources. Altogether 123 31 antibiotic-resistant isolates belonging to 16 different S. enterica serovars were classified into 32 3 groups according to the presence of SGI1 and the presence of integrons. The first group 33 consisted of 62 strains in which neither SGI1 nor class 1 integron was detected. A high 34 diversity among serovars and resistance phenotypes was found in this group. The second 35 group consisted of 56 strains positive for both the SGI1 and class 1 integron, out of which 55 36 belonged to serovar Typhimurium and one to a nonmotile serovar [4,12] which harboured the 37 SGI1-B variant. The third group comprised five strains which were positive for class 1 38 integron but negative for the SGI1. Sequencing of the integrons in these isolates identified 39 integron with sat1 and aadA1 gene cassettes in S. Sandiego and S. Pullorum, dfrA1 and aadA1 40 gene cassettes in S. Typhimurium integron, and *aadA21* gene cassette in S. Braenderub and S. 41 Zanzibar. 42 43 Keywords 44 Salmonella, antibiotic resistance, SGI1, integron, plasmid

45

46 Introduction

47 The failure of bacterial disease treatment due to the antibiotic resistance of pathogenic

- 48 microorganisms is a cause for concern for both the scientific community and the general
- 49 public. Pathogenic microorganisms may become resistant to antibiotics in multiple ways. The
- 50 two most extreme examples are point mutations in house-keeping genes leading to the

resistance phenotype or the horizontal acquisition of genes coding for antibiotic resistance. In *Salmonella enterica* (*S. enterica*), an example of the former case is the resistance to nalidixic
acid which is most frequently associated with point mutations in the *gyrA* gene (Reyna et al.,
1995). An example of the latter case is the appearance of multidrug resistant *S*. Typhimurium
which acquired the *Salmonella* Genomic Island 1 (SGI1), part of which codes for resistance to
antibiotics (Boyd et al., 2001).

57

58 In S. enterica, strains belonging to serovar Typhimurium are most frequently reported to be 59 antibiotic resistant and within this serovar, one dominant lineage can be identified which is 60 comprised of highly similar strains with the chromosomally located SGI1 (Boyd et al., 2001; 61 Matiasovicova et al., 2007). Such strains were first recorded in the mid 1980's in the UK 62 (Threlfall et al., 1994) and since that time they have spread throughout the whole world. Why this has happened is not fully understood but explanations regarding their increased virulence 63 64 or ability of biofilm formation are being sought (Evans and Davies, 1996; Malcova et al., 65 2008). The remaining antibiotic resistant strains of S. Typhimurium are highly heterogenous 66 and consist mainly of strains in which different conjugative R-plasmids can be found 67 (Pezzella et al., 2004; Rodriguez et al., 2006; Vo et al., 2007).

68

69 Unlike some extensive studies, aim of our work was to analyse the relative frequency of 70 different modes of antibiotic resistance in a collection of strains all isolated during a single 71 year from animal sources from a relatively restricted geographic area. We therefore tested *S*. 72 *enterica* strains which were submitted for confirmation of their antibiotic resistance to the 73 National Reference Laboratory for Antibiotic Resistance in the Czech Republic over the 74 course of a single year. This approach gave us a realistic view of the current situation among 75 antibiotic resistant strains of *S. enterica* of animal origin in the Czech Republic regardless of

- the serovar which, due to similarities in technologies of animal production, can be with acertain care generalised for the rest of Central Europe.
- 78

79 Materials and Methods

80 Bacterial strains, serotyping and antibiotic susceptibility testing

- 81 A total of 123 Salmonella enterica strains isolated from animal sources in the Czech Republic 82 during year 2006 were included in this study. The strains were submitted to our laboratory by 83 local diagnostic laboratories because of their resistance to one or more antibiotics. The strains 84 originated from pigs (n=61), cattle (n=18), chickens (n=11), turkeys (n=6) and from other 85 animal or environmental sources (n=27) (for additional details see supplementary material). 86 Isolated Salmonella were typed by agglutination with monovalent O and H anti-Salmonella 87 sera (Bio-Rad, France), and classified into serotypes according to the Kauffman-White 88 system. All isolates were tested by the disc diffusion method based on CLSI standards for sensitivity to the following 18 antibiotics (Oxoid Ltd., UK): ampicillin (A, 10 µg), 89 90 amoxycillin/clavulanic acid (AMC, 30 µg), apramycin (APR, 15 µg), colistin (CT, 10 µg), 91 cefotaxime (CTX, 30 µg), cephalothin (KF, 30 µg), ceftiofur (EFT, 30 µg), ciprofloxacin 92 (CIP, 5 μ g) chloramphenicol (C, 30 μ g), enrofloxacin (ENR, 5 μ g), gentamicin (G, 10 μ g), 93 kanamycin (K, 30 μg) nalidixic acid (NA, 30 μg), neomycin (N, 30 μg), streptomycin (S, 10 94 μ g), tetracycline (T, 30 μ g), sulphonamides (Su, 300 μ g) and trimethoprim (W, 5 μ g). 95
- 96 PCR detection of the left junction of SGI1 and class 1 integrons
- 97 All the strains were tested for the presence of *Salmonella* genomic island 1 (SGI1) and class 1
- 98 integrons. PCR for the detection of the left junction of SGI1 was carried out with the primer
- 99 pair U7-L12 and LJ-R1 and the whole integron sequence was amplified using the 5CS and
- 100 3CS primer pair described previously (Levesque et al., 1995; Boyd et al., 2002). PCR
- 101 conditions we described previously (Faldynova et al., 2003).

102

103 Sequencing of class 1 integrons

104	To identify gene cassettes inside the class 1 integrons, the resulting PCR products were
105	sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit according to the
106	instructions of the manufacturer (Applied Biosystems). The extension products were
107	separated and analysed by the ABI 310 Genetic Analyser (Applied Biosystems) and obtained
108	sequences were BLAST compared with GenBank entries. The sequencing was performed for
109	all strains which were different from S. Typhimurium displaying the ACSSuT or
110	ACSSuT+Nal resistance phenotype and giving rise to 2 expected PCR products of 1009 and
111	1197 bp after the 5CS-3CS PCR, or S. Typhimurium displaying the SSu resistance phenotype
112	and giving rise to a single 1009 bp PCR product after the 5CS-3CS PCR. Such isolates were
113	considered to contain either SGI1 or the SGI1-C variant of SGI1 (Boyd et al., 2002).
114	
115	Results
116	Serotyping and antibiotic susceptibility testing
117	The 123 antibiotic-resistant Salmonella isolates were assigned to 16 different S. enterica

serovars including Typhimurium (n=93), Enteritidis (n=6), Infantis (n=5), Derby (n=4),

119 Choleraesuis (n=2), Saintpaul (n=2) and Zanzibar (n=2). One isolates each was classified as

120 belonging to the serovars Braenderub, Kentucky, Langeveld, Lomita, London, Ohio,

121 Sandiego, Uppsala and a nonmotile serovar [4,12].

122

123 Resistance to streptomycin, ampicillin and sulphonamides was most frequently observed (in

approx. 83 % of all isolates), followed by resistance to tetracycline (74 %) and

125 chloramphenicol (54.5 %). The rates of resistances to nalidixic acid, trimethoprim and

126 kanamycin were 13.8 %, 7.3 % and 4.1 %, respectively. Only 2 strains (1.6 %) were resistant

127 to neomycin or cefalotin. Resistance to apramycin, gentamicin or amoxycilin-clavulanic acid

128 was always observed in a single isolate. Within the given collection of strains and the set of

129 antibiotics used we never observed strains resistant to cefotaxime, ceftiofur, ciprofloxacin,

130 colistin and enrofloxacin.

131

132 PCR detection of the left junction of SGI1 and class 1 integrons

133 Based on the two PCRs, the strains could be clustered into three groups. The first group

134 consisted of 62 strains in which neither the left junction of SGI1 nor the class 1 integron was

135 detected. A high diversity in serovars and resistance phenotypes was found in this group (see

136 Table 1 and also electronic supplementary material). This type of antibiotic resistance was

137 most frequently found in serovar Typhimurium (59.7 %). Although the isolates were resistant

138 from 1 to 8 antibiotics, the most frequent type of resistance of non-SGI1 *S*. Typhimurium was

139 characterised as ASSuT.

140

The second group consisted of 56 strains positive for both the left junction of SGI1 and the class 1 integron. Except for a single isolate of nonmotile *Salmonella* [4,12] isolated from a shark, all the strains in this group belonged to serovar Typhimurium. The nonmotile *Salmonella* [4,12] strain was resistant to ampicillin and sulphonamides and both these resistances were associated with the presence of the SGI1-B variant present downstream from the *thdF* gene.

147

148 The third group comprised five strains of serovars Braenderub (SSuT resistance phenotype),

149 Sandiego (SSuT), Saintpaul (SSuT), Typhimurium (ASSuT+W+Apr) and Zanzibar (CSSuT)

150 which were positive for the class 1 integron but negative for the left junction of SGI1. To

- 151 determine which of the antibiotic resistances were parts of the integrons, the 5CS-3CS PCR
- 152 products were subjected to DNA sequencing.
- 153
- 154 Sequencing of class 1 integrons
- 155 In the 5 strains in which the integrons were not part of the SGI1 and, S. Sandiego and S.
- 156 Saintpaul harboured integrons with *sat1* and *aadA1* gene cassettes similar to the GenBank
- 157 Ac.n. AM055748. S. Typhimurium harboured integron with dfrA1 and aadA1 gene cassettes
- similar to the GenBank Ac.n. DQ133164, and in S. Zanzibar and S. Braenderub, an *aadA21*
- 159 containing integron similar to the GenBank Ac.n. AY171244 was found. All these gene
- 160 cassettes were different from those usually found in different SGI1 variants further
- 161 confirming the non-SGI1 nature of these integrons (Table 2).
- 162

163 **Discussion**

- 164 The S. enterica isolates in this study were most frequently resistant to streptomycin,
- 165 ampicillin and sulphonamides. Resistance to tetracycline and chloramphenicol were recorded
- 166 slightly less frequently (Fig. 1). The dominant occurrence of these resistances is closely
- 167 linked with the presence of the SGI1 and S. Typhimurium. Indeed, in 56 isolates with the
- 168 ACSSuT type of resistance all belonged to the serovar Typhimurium and only 4 of them did
- 169 not harbour the SGI1. Less frequent resistance to chloramphenicol can be explained by the
- 170 detection of 19 strains without the SGI1 (18 isolates of serovar Typhimurium and a single
- 171 isolate of serovar Infantis) which were resistant to ampicillin, streptomycin, sulphonamides
- and tetracycline. Isolates of S. Typhimurium exhibiting this type of resistance have already
- been recorded by other authors (Soler et al., 2006; Graziani et al., 2008).
- 174

175	Sixty two S. enterica isolates resistant to antibiotics were free of the SGI1 as well as
176	integrons. The increased variation in serovars represented in this group indicated a recent
177	horizontal acquisition of antibiotic resistance from different sources. It is possible that at least
178	in some of the cases the resistance was encoded by conjugative plasmids which may transfer
179	resistance to a single antibiotic (Hradecka et al., 2006) as well as multidrug resistance
180	(Carattoli et al., 2001; Guerra et al., 2002).
181	
182	The SGI1 and integrons were found in 56 isolates, 55 of which belonged to serovar
183	Typhimurium. The only non-Typhimurium serovar harbouring the SGI1 was nonmotile
184	Salmonella [4,12] with a SGI1-B variant (ASu type of antibiotic resistance). Although the
185	SGI1 can be mobilised for conjugative transfer (Doublet et al., 2005), its dominant association
186	with serovar Typhimurium and much less frequent presence in other serovars also suggests
187	that the conjugational transfer of SGI1 is probably not a common event in the environment.
188	
189	Five strains were positive for the presence of integrons but negative for the SGI1. Since these
190	strains were all from different serovars, it is quite probable that the integrons were acquired
191	by each of the isolates independently. All combinations of gene cassettes identified in our
192	strain collection have already been described although the sat1 aadA1 combination is found in
193	S. enterica quite rarely (Guerra et al., 2000; Zhao et al., 2007).
194	
195	Although antibiotic resistance testing is a relatively simple procedure, a detailed
196	characterisation of genotypes of antibiotic resistant S. enterica by PCR targeted to the
197	antibiotic resistance genes is quite laborious due to the extensive list of potential gene

- 198 candidates (Faldynova et al., 2003). The last, rather indirect result of this study therefore is
- 199 that by a combination of only two PCRs specific for the left junction of the SGI and the

200	presence of integrons, S. enterica isolates can be easily classified into 3 basic clusters. The
201	sizes of the amplification products in 5CS-3CS PCR enabled the further characterisation of
202	the field isolates and the selection of strains with unusual characteristics suitable for
203	subsequent detailed analysis.
204	
205	Acknowledgement
206	This work has been supported by projects 1B44019 and MZE0002716201 of the Czech
207	Ministry of Agriculture. The authors wish to acknowledge the excellent technical assistance
208	of Michaela Dekanova and Neysan Donelli for English language corrections.
209	
210	References
211	Boyd, D., Cloeckaert, A., Chaslus-Dancla, E., Mulvey, M.R., 2002. Characterization of
212	variant Salmonella genomic island 1 multidrug resistance regions from serovars Typhimurium
213	DT104 and Agona. Antimicrob. Agents Chemother. 46, 1714-1722.
214	
215	Boyd, D., Peters, G.A., Cloeckaert, A., Boumedine, K.S., Chaslus-Dancla, E., Imberechts, H.,
216	Mulvey, M.R., 2001. Complete nucleotide sequence of a 43-kilobase genomic island
217	associated with the multidrug resistance region of Salmonella enterica serovar Typhimurium
218	DT104 and its identification in phage type DT120 and serovar Agona. J. Bacteriol. 183, 5725-
219	5732.
220	
221	Carattoli, A., Villa, L., Pezzella, C., Bordi, E., Visca, P., 2001. Expanding drug resistance
222	through integron acquisition by IncFI plasmids of Salmonella enterica Typhimurium. Emerg.
223	Infect. Dis. 7, 444-447.
224	

- 225 Doublet, B., Boyd, D., Mulvey, M.R., Cloeckaert, A., 2005. The Salmonella genomic island 1
- is an integrative mobilizable element. Mol. Microbiol. 55, 1911-1924.
- 227
- 228 Evans, S., Davies, R., 1996. Case control study of multiple-resistant Salmonella typhimurium
- 229 DT104 infection of cattle in Great Britain. Vet. Rec. 139, 557-558.
- 230
- 231 Faldynova, M., Pravcova, M., Sisak, F., Havlickova, H., Kolackova, I., Cizek, A.,
- 232 Karpiskova, R., Rychlik, I., 2003. Evolution of antibiotic resistance in Salmonella enterica
- serovar typhimurium strains isolated in the Czech Republic between 1984 and 2002.
- Antimicrob. Agents Chemother. 47, 2002-2005.
- 235
- 236 Graziani, C., Busani, L., Dionisi, A.M., Lucarelli, C., Owczarek, S., Ricci, A., Mancin, M.,
- 237 Caprioli, A., Luzzi, I., 2008. Antimicrobial resistance in Salmonella enterica serovar
- Typhimurium from human and animal sources in Italy. Vet. Microbiol. 128, 414-418.
- 239
- 240 Guerra, B., Soto, S., Cal, S., Mendoza, M.C., 2000. Antimicrobial resistance and spread of
- 241 class 1 integrons among Salmonella serotypes. Antimicrob. Agents Chemother. 44, 2166-
- 242 2169.
- 243
- 244 Guerra, B., Soto, S., Helmuth, R., Mendoza, M.C., 2002. Characterization of a self-
- transferable plasmid from *Salmonella enterica* serotype typhimurium clinical isolates carrying
- two integron-borne gene cassettes together with virulence and drug resistance genes.
- 247 Antimicrob. Agents Chemother. 46, 2977-2981.

248

249	Hradecka.	Η.	Kolackova.	Ι.	Kar	piskova.	R.	Ry	chlik.	I.	2006.	An	outbreak	of h	uman
	I II a a c c II a,		, itoiaono , a.		, I LOUI	piblic ra.	, <u></u>		viiii.	, .	, =0000.		outoroun	UI 11	aman

250 salmonellosis caused by ampicillin-resistant Salmonella enterica serovar Enteritidis PT13 in

- 252
- Levesque, C., Piche, L., Larose, C., Roy, P.H., 1995. PCR mapping of integrons reveals
- several novel combinations of resistance genes. Antimicrob. Agents Chemother. 39, 185-191.

255

- 256 Malcova, M., Hradecka, H., Karpiskova, R., Rychlik, I., 2008. Biofilm formation in field
- 257 strains of Salmonella enterica serovar Typhimurium: Identification of a new colony
- morphology type and the role of SGI1 in biofilm formation. Vet. Microbiol. 129, 360-6.

259

- 260 Matiasovicova, J., Adams, P., Barrow, P.A., Hradecka, H., Malcova, M., Karpiskova, R.,
- 261 Budinska, E., Pilousova, L., Rychlik, I., 2007. Identification of putative ancestors of the
- 262 multidrug-resistant Salmonella enterica serovar typhimurium DT104 clone harboring the
- 263 Salmonella genomic island 1. Arch. Microbiol. 187, 415-424.
- 264
- 265 Pezzella, C., Ricci, A., DiGiannatale, E., Luzzi, I., Carattoli, A., 2004. Tetracycline and
- 266 streptomycin resistance genes, transposons, and plasmids in Salmonella enterica isolates from

animals in Italy. Antimicrob. Agents Chemother. 48, 903-908.

- 268
- 269 Reyna, F., Huesca, M., Gonzalez, V., Fuchs, L.Y., 1995. Salmonella typhimurium gyrA
- 270 mutations associated with fluoroquinolone resistance. Antimicrob. Agents Chemother. 39,
- 271 1621-1623.

272

the Czech Republic. Epidemiol. Infect. 134, 737-740.

- 273 Rodriguez, I., Martin, M.C., Mendoza, M.C., Rodicio, M.R., 2006. Class 1 and class 2
- 274 integrons in non-prevalent serovars of Salmonella enterica: structure and association with

276

- 277 Soler, P., Gonzalez-Sanz, R., Bleda, M.J., Hernandez, G., Echeita, A., Usera, M.A., 2006.
- 278 Antimicrobial resistance in non-typhoidal *Salmonella* from human sources, Spain, 2001-2003.
- 279 J. Antimicrob. Chemother. 58, 310-314.

280

- 281 Threlfall, E.J., Frost, J.A., Ward, L.R., Rowe, B., 1994. Epidemic in cattle and humans of
- 282 Salmonella typhimurium DT 104 with chromosomally integrated multiple drug resistance.
- 283 Vet. Rec. 134, 577.
- 284
- 285 Vo, A.T., van Duijkeren, E., Fluit, A.C., Gaastra, W., 2007. A novel Salmonella genomic
- island 1 and rare integron types in *Salmonella* Typhimurium isolates from horses in The
- 287 Netherlands. J. Antimicrob. Chemother. 59, 594-599.

288

- 289 Zhao, S., McDermott, P.F., White, D.G., Qaiyumi, S., Friedman, S.L., Abbott, J.W., Glenn,
- A., Ayers, S.L., Post, K.W., Fales, W.H., Wilson, R.B., Reggiardo, C., Walker, R.D., 2007.
- 291 Characterization of multidrug resistant *Salmonella* recovered from diseased animals. Vet.
- 292 Microbiol. 123, 122-132.

transposons and plasmids. J. Antimicrob. Chemother. 58, 1124-1132.

293 Figure legend

- 294 Figure 1. Resistance to individual antibiotics among *S. enterica* strains isolated from animal
- sources in the Czech Republic during 2006. A, ampicillin; AMC, amoxycillin/clavulanic acid;
- 296 APR, apramycin; C, chloramphenicol, G, gentamicin; K, kanamycin; KF, cefalotin; N,
- 297 neomycin; NA, nalidixic acid; S, streptomycin; Su, sulphonamides; T, tetracycline; W,
- trimethoprim.

- 299 Table 1. Variability in serovars and antibiotic resistance patterns depending on the presence of
- 300 SGI1 or integrons

group	LJ ^{&}	5CS 3CS ^{&}	strains*	serovars*	resistance*
1	-	-	62	13	27
2	+	+	56	2	5
3	-	+	5	5	3

- 301
- 302 * numbers indicate number of strains, serovars or different resistance patterns in each group
- 303 [&] result of particular PCR

- 304 Table 2. Integrons of *Salmonella enterica* as identified by DNA sequencing of 5CS-3CS PCR
- 305 products.
- 306

Serovar	Resistance	Presence	Array of gene cassettes	Origin
	phenotype	of SGI1		
[4,12]	ASu	yes	bla _{PSE-1}	shark
Braenderub	SSuT	no	aadA21	chicken
Sandiego	SSuT	no	sat1 aadA1	turkey
Saintpaul	SSuT	no	sat1 aadA1	turkey
Typhimurium	ASSuT+W+Apr	no	dfrA1 aadA1	pig
Zanzibar	CSSuT	no	aadA21	turkey

307

308 A, ampicillin; Apr, apramycin; C, chloramphenicol; S, streptomycin; Su, sulphonamides; T,

309 tetracycline; W, trimethoprim

Fig 1. Havlickova et al. Distribution of integrons and SGI1 among antibiotic resistant Salmonella...

