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A novel *Salmonella* genomic island 1 and rare integron types in *Salmonella* Typhimurium isolates from horses in The Netherlands

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***Objectives*: To investigate the genotypic resistance of integron-carrying *Salmonella* Typhimurium isolates from horses and their genetic relationship.**

***Methods*: Sixty-one *Salmonella* isolates were screened for the presence of class 1 integrons by PCR. The gene cassettes of integron-positive isolates were detected by PCR, restriction fragment length polymorphism typing, and sequencing. The potential for the transfer of resistance determinants was investigated by conjugation experiments. The presence of *Salmonella* genomic island 1 (SGI1) or its variants was studied by PCR and nucleotide sequencing. PFGE was used to genotype the isolates.**

***Results*: Eight distinct *Xba*I-PFGE profiles and seven integron types were observed among 26 integron-carrying *Salmonella* Typhimurium isolates. The gene cassettes detected were *dfrA1*, *dfrA7*, *dfrA14*, *aadA1*, *aadA2*, *aadB* and bla_{PSE}. A rare type of integron found in nine isolates carried the *dfrA14* and *aadA1* gene cassettes. Twelve *Salmonella* Typhimurium DT104 isolates contained SGI1 or one of its variants (SGI1, SGI1-B and SGI1-C). A novel variant of SGI1, designated SGI1-M, was identified in one isolate in which the *aadA2* gene of SGI1 was replaced by the *aadB* gene. Transfer of integrons and antimicrobial resistance determinants to *Escherichia coli* K12 via conjugation was possible with nine isolates. Resistance to fluoroquinolones in nine isolates was caused by mutations in the *gyrA* gene leading to the amino acid changes Ser-83 → Ala and Asp-87 → Asn.**

***Conclusions*: The integron-positive clinical *Salmonella* Typhimurium isolates from horses belong to distinct strains. The data demonstrate the capability of *Salmonella* Typhimurium to acquire additional antibiotic resistance determinants and underline the need for the prudent use of antimicrobials.**

Keywords: *Salmonella* spp., genotyping, SGI1, multidrug resistance, conjugation

Introduction

Over the last decade, a large increase in multidrug-resistant (MDR) *Salmonella enterica* isolates has been documented.[1] In humans, *Salmonella* Typhimurium is a major aetiological agent of food-borne salmonellosis. In The Netherlands, *Salmonella* Typhimurium is the predominant serovar causing salmonellosis in horses, and this serovar was more often resistant to antimicrobial agents when compared with other *Salmonella* serovars.[2] MDR *Salmonella* isolates from horses can be transferred to humans by direct contact or indirectly through the food chain.[3]

Multidrug resistance is strongly linked to the presence of class 1 integrons.[4] Integrons are genetic elements that recognize and capture mobile gene cassettes (often encoding antibiotic resistance) by site-specific recombination.[5] Three classes of integrons have been described. Class 1 integrons are the most common integrons found in clinical *Salmonella* isolates.[6] *Salmonella* genomic island 1 (SGI1) is a 43 kb genomic island, which contains a complex integron.[7] Variants of SGI1 (A–L) have been found in several *Salmonella* serovars including *Salmonella* Typhimurium.[8–10] SGI1 can be transferred to other bacteria like *Escherichia coli* in the presence of a helper plasmid.[11] Van Duijkeren *et al.*[12] described that a particular type of integron, which was identified by restriction fragment length polymorphism (1600 bp, type XIII), was exclusively detected in equine *Salmonella* Typhimurium isolates. This observation prompted the current investigation.

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In the present study we investigated (i) the genetic relationship of 26 clinical isolates of equine integron-carrying MDR *Salmonella* Typhimurium, (ii) the characteristics of the antimicrobial resistance determinants among the isolates, and (iii) the potential to transfer these antibiotic resistance determinants to other bacterial species.

Materials and methods

Bacterial isolates

During the period 1993–2005, a total of 406 clinical equine *Salmonella* isolates was collected by the Veterinary Microbiological Diagnostic Center (VMDC) of Utrecht University, The Netherlands. The isolates were identified as *Salmonella* by biochemical testing and further characterized by serotyping. Based on the susceptibility testing data which were recorded by the VMDC, 61 *Salmonella* group B isolates from this collection were selected, based on their resistance to at least one antimicrobial drug. All isolates were cultured from different horses that belonged to different owners living throughout the country. The 61 isolates were screened for the presence of class 1 integrons using PCR amplification of the class 1 integrase gene.[13] The 26 integron-carrying *Salmonella* Typhimurium isolates were phage-typed using the Dutch phage-typing system.[14] Phage type 506 in this system corresponds to phage type DT104 in the English phage-typing system.[15] The 26 isolates (Table 1), including 8 isolates described in a previous study,[12] were tested for their antimicrobial susceptibility by the disc diffusion assay using Neo-Sensitab discs (Rosco, Denmark) based on the procedure recommended by the Dutch Committee on Guidelines for Susceptibility Testing CRG.[16] The antimicrobials tested were ampicillin (30 μg), amoxicillin/clavulanic acid (30/15 μg), cefalexin (30 μg), ceftiofur (30 μg), flumequine (30 μg), enrofloxacin (10 μg), streptomycin (100 μg), gentamicin (40 μg), kanamycin (100 μg), chloramphenicol (60 μg), tetracycline (80 μg), and trimethoprim/sulfamethoxazole (5.2/240 μg). In addition, the isolate H37 was tested for its susceptibility to tobramycin (40 μg) since its integron contained the *aadB* gene encoding resistance to aminoglycosides.

Gene cassette characterization

The gene cassettes inserted in the integrons of the isolates were determined by PCR with primers for the conserved segment regions (CS-PCR).[17] CS-PCR amplicons of the same size were subjected to restriction fragment length polymorphism (RFLP) typing and were considered identical if they had the same RFLP pattern after digestion with at least two enzymes (Table 1). The RFLP patterns obtained were compared with those from a previous study.[18] If these data were not available, a representative of each RFLP type was randomly chosen for nucleotide sequencing on an ABI 3730 Sequencer. The obtained nucleotide sequences have been deposited in GenBank (accession numbers DQ388123, DQ388124, DQ388125 and DQ388126).

Detection of SGI1 and its variants

All 26 isolates were examined for the presence of the left and right junction of SGI1. The presence of sequences from the antibiotic resistance gene cluster was determined by PCR as described previously.[10,19,20] Three *Salmonella* Typhimurium isolates carrying SGI1, SGI1-B and SGI1-C, respectively,[18] were included as controls. To confirm the gene identity and the linkage between genes, the products generated by PCR mapping (Figure 1) were either sequenced or cloned using DNA of *Salmonella* Typhimurium isolate H37 as template.

The two transconjugants obtained from the conjugation of isolate H16 and H18 and *E. coli* were examined for the insertion of SGI1 or SGI1-C into their chromosome. PCR assays were performed as described previously.[11] If SGI1 or SGI1-C can be transferred from *Salmonella* H16 or H18, respectively, and inserted into the *E. coli* genome, we would expect that SGI1 will be flanked by the *thdF* gene and the *tnaL* gene of the *E. coli* transconjugants.[11]

Detection of gyrA mutations by allele-specific (AS)-PCR-RFLP assay and nucleotide sequencing

Nine enrofloxacin-resistant *Salmonella* Typhimurium isolates were tested by AS-PCR and RFLP analysis[21] to detect mutations in codons 81, 83 and 87 of the *gyrA* gene. When more than one isolate had the same RFLP pattern, one representative fragment was chosen for nucleotide sequencing. The enrofloxacin-resistant isolates were also tested for the presence of the *qnrA1* gene by PCR.[22]

Bacterial conjugation and plasmid analysis

A conjugation experiment was performed to determine whether the integrons and resistance determinants of the 26 *Salmonella* Typhimurium isolates could be transferred to *E. coli*. A rifampicin-resistant and sulfamethoxazole-susceptible *E. coli* K12 strain was used as the recipient as described previously.[23] The transconjugants were tested for their biochemical characteristics by the API 20E system (bioMérieux, Marcy-l'Etoile, France) and their susceptibility patterns and integrons were determined as described above. In addition, plasmid analysis was performed using the phenol–chloroform extraction procedure[24] for both the *Salmonella* donors and the *E. coli* transconjugants. The reference strain was a *Salmonella* Typhimurium phage type 13 strain containing five plasmids ranging in size between 4.4 and 180 kb.[23]

Pulsed field gel electrophoresis

To determine the genetic relationship among the 26 integron-carrying *Salmonella* Typhimurium isolates, PFGE analysis was performed as described previously.[23] The reference isolates were PulseNet *Salmonella* Braenderup and *Salmonella* Senftenberg. PFGE profiles were defined as different when their PFGE patterns had at least one band difference.

Southern-blot hybridization

To determine whether integrons were located at the same position of the *Salmonella* Typhimurium genome for all isolates, Southern blot hybridization was performed by the capillary blot procedure using the nine enrofloxacin-resistant isolates. A luminescent DIG labelling and detection kit (Roche, Mannheim, Germany) was used according to the manufacturer's instructions.

Results

Twenty-six (43%) of the *Salmonella* Typhimurium isolates carried at least one integron. The phage types, resistance phenotypes, characteristics of inserted gene cassettes, SGI1 types, *Xba*I-PFGE profiles, and the results of the conjugation experiments are summarized in Table 1. The integron-carrying isolates belonged to 8 different phage types, had 9 different phenotypic resistance profiles and were resistant to 1–9 antimicrobial agents. Eight PFGE profiles were defined (Table 1 and Figure 2). Seven integron types were found. Nine *Salmonella* Typhimurium isolates of different

Table 1. Antimicrobial resistance characteristics of clinical equine *Salmonella* Typhimurium isolates in The Netherlands

ID no.	Year	Phage type	Resistance phenotype	Cassette size (in bp)	Gene cassette	SGI1 type	*Xba*I-PFGE	GyrA change	Conj.	*E. coli* transconjugants		
										int	resistance type	plasmid (kb)
H1	95	RDNC	ACSSuTEFGK	1600	*dfrA14*, *aadA1*	–	I	+[a]	+	+	ACSTSuRif	not detected
H2	95	RDNC	ACSSuTEFGK	1600	*dfrA14*, *aadA1*	−	I	+[a]	−			
H3	95	351	ACSSuTEFGK	1600	*dfrA14*, *aadA1*	−	II	+[a]	−			
H4	95	UT	ACSSuTEFGK	1600	*dfrA14*, *aadA1*	−	I	+[a]	−			
H5	95	508	ACSSuTEFGK	1600	*dfrA14*, *aadA1*	−	II	+[a]	+	+	ACSTSuRif	not detected
H6	96	353	ACSSuTEFGK	1600	*dfrA14*, *aadA1*	−	I	+[a]	−			
H7	96	150	ACSSuTEFGK	1600	*dfrA14*, *aadA1*	−	I	+[a]	+	+	ACSTSuRif	45
H38	96	150	ACSSuTEFGK	1600	*dfrA14*, *aadA1*	−	I	+[a]	−			
H8	99	508	ACSSuTEFGK	1600	*dfrA14*, *aadA1*	−	I	+[a]	+	+	ASTSuRif	90; 3.7
H36	97	RDNC	ACGSuT	1600	*dfrA1*, *aadA1*	−	V	NT	+	+	ACTSuRif	90
H27	03	RDNC	ASSuT	1600	*dfrA1*, *aadA1*	−	VI	NT	+	+	ASTSuRif	90; 6.6
H16	01	DT104	ACSSuT	1000; 1200	*aadA2*, bla_{PSE-1}	SGI1	III	NT	+	+	ASSuRif	90; 6.6; 3.7
H17	01	DT104	ACSSuT	1000; 1200	*aadA2*, bla_{PSE-1}	SGI1	IV	NT	−			
H19	01	DT104	ACSSuT	1000; 1200	*aadA2*, bla_{PSE-1}	SGI1	III	NT	−			
H20	01	DT104	ACSSuT	1000; 1200	*aadA2*, bla_{PSE-1}	SGI1	III	NT	−			
H22	01	DT104	ACSSuT	1000; 1200	*aadA2*, bla_{PSE-1}	SGI1	III	NT	−			
H23	01	DT104	ACSSuT	1000; 1200	*aadA2*, bla_{PSE-1}	SGI1	III	NT	−			
H24	01	DT104	ACSSuT	1000; 1200	*aadA2*, bla_{PSE-1}	SGI1	III	NT	−			
H25	01	DT104	ACSSuT	1000; 1200	*aadA2*, bla_{PSE-1}	SGI1	III	NT	−			
H26	01	DT104	ACSSuT	1000; 1200	*aadA2*, bla_{PSE-1}	SGI1	IV	NT	−			
H35	05	DT104	ACSSuT	1000; 1200	*aadA2*, bla_{PSE-1}	SGI1	III	NT	−			
H37	95	DT104	AAcCSSuGKTo	800; 1200	*aadB*, bla_{PSE-1}	SGI1-M	III	NT	−			
H39	93	RDNC	ACSSuTKF	800	*dfrA7*	−	VIII	NT	+	+	ACSTSuRif	not detected
H40	93	204	ACSSuTKF	800	*dfrA7*	−	VII	NT	−			
H18	01	DT104	ASSu	1000	*aadA2*	SGI1-C	III	NT	+	+	ASuRif	90
H29	03	DT104	A	1200	bla_{PSE-1}	SGI1-B	IV	NT	−			

Conj., conjugation; *int*, integrase gene; RDNC, reaction does not correspond to any recognized phage types; UT, untypeable; NT, not tested; A, ampicillin; Ac, amoxicillin/clavulanic acid; C, chloramphenicol; S, streptomycin; G, gentamicin; K, kanamycin; T, tetracycline; Su, trimethoprim/sulfamethoxazole; E, enrofloxacin, F, flumequine; Rif, rifampicin; To, tobramycin.

[a]Amino acid changes: Ser-83 → Ala and Asp-87 → Asn.

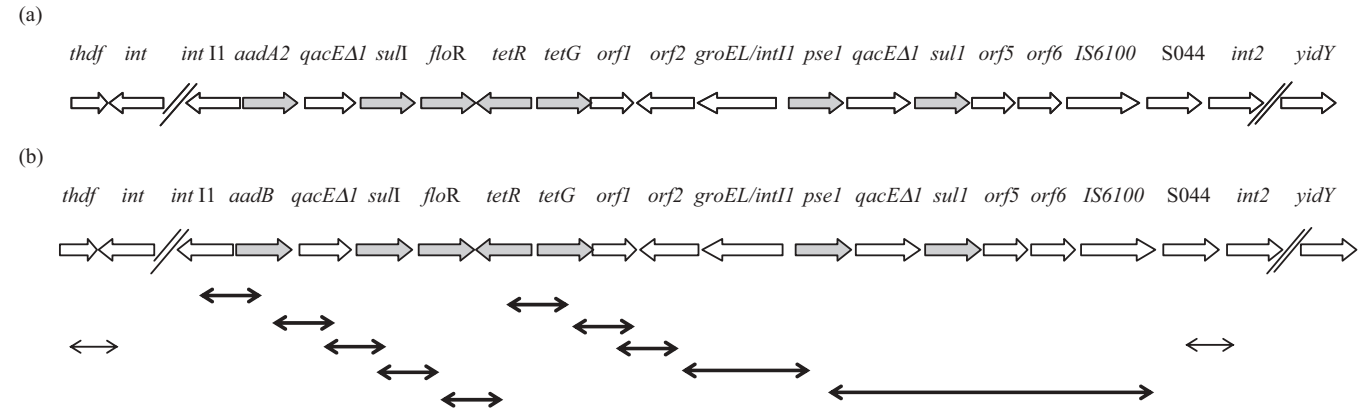

Figure 1. (a) Map of SGI1 adapted from Boyd *et al.*[8] (b) Map of SGI1-M highlighting targets in PCRs used for mapping SGI1 antibiotic resistance gene clusters found in *Salmonella* Typhimurium (H37). Sequences of fragments indicated by bold arrows were confirmed by sequencing. Grey shading indicates antibiotic resistance genes.

phage types with resistance pattern ACSSuTEFGK and integron type XVI (*dfrA14*, *aadA1*), were grouped in PFGE profile I or II. The 13 *Salmonella* Typhimurium DT104 isolates were grouped in PFGE profiles III or IV. Ten of these isolates had the resistance phenotype ACSSuT and contained a type I integron (*aadA2*, bla_{PSE-1}). The two isolates that were non-typeable by phages and carried a type VII integron (*dfrA1*, *aadA1*) were classified into PFGE profiles V or VI. The two isolates of phage type 204 and RDNC, respectively, both with integron type XVIII (*dfrA7*), were classified into profile VII or VIII.

The cassettes present in the integrons carried the *aadA1*, *aadA2* and *aadB* genes encoding resistance to aminoglycosides; the *dfrA1*, *dfrA7* and *dfrA14* genes conferring resistance to trimethoprim, and the bla_{PSE-1} gene encoding resistance to ampicillin. Nine *Salmonella* Typhimurium isolates carried an integron with the *dfrA14–aadA1* gene cassettes. Southern blot hybridization with an integrase-specific probe showed that the integrons in eight of nine isolates tested hybridized to similar sized fragments suggesting that these integrons have a similar position on the chromosome.

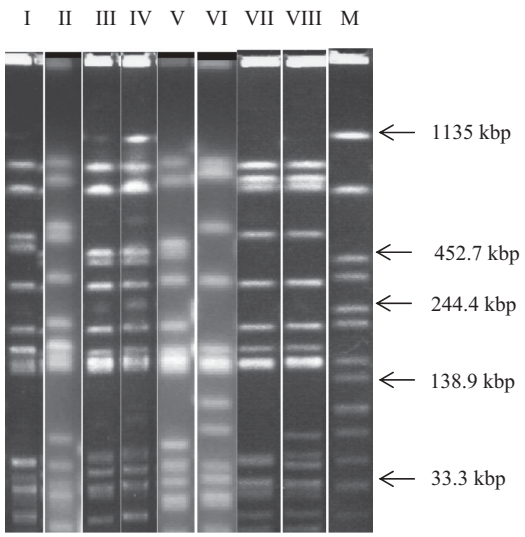

Figure 2. *Xba*I-PFGE profiles (I–VIII) of integron-carrying *Salmonella* Typhimurium isolates from horses in The Netherlands. M, marker strain, *Salmonella* Braenderup.

Ten *Salmonella* Typhimurium DT104 isolates contained SGI1, one isolate carried SGI1-B and one isolate contained SGI1-C (Table 1). Based on the nucleotide sequencing results, the antibiotic resistance cluster in isolate H37 (Figure 1) is part of a new SGI1 variant for which we propose the name SGI1-M. The *aadA2* gene encoding resistance against spectinomycin and streptomycin of SGI1 and other variants (SGI1-A, -C, -D, -E, -I) was replaced in this variant by the *aadB* gene encoding kanamycin, gentamicin and tobramycin resistance. The isolate H37 indeed showed phenotypic resistance to kanamycin, gentamicin and tobramycin.

The nine enrofloxacin-resistant *Salmonella* Typhimurium isolates had mutations in the two codons for amino acids at positions 83 and 87 of the *gyrA* gene as shown by AS-PCR-RFLP. Nucleotide sequence analysis confirmed that mutations were present at codons 83 and 87 where nucleotides TCC and GAC were replaced by GCC and AAC, respectively, leading to amino acid substitutions Ser-83 → Ala and Asp-87 → Asn. No *qnrA1*-carrying isolate was found.

Integrase amplification using genomic DNA of the transconjugants indicated that nine *Salmonella* Typhimurium isolates belonging to different phage types could transfer integrons to *E. coli* (Table 1). The antibiotic resistance phenotype of the transconjugants and the sizes of the plasmids detected are shown in Table 1. It should be noted that a large 90 kb plasmid was found in half of the transconjugants. However, no plasmid was detected in some transconjugants, although they had a resistance phenotype (ACSSuTRif) similar to that of the donor.

The isolates H16 and H18, which carried SGI1 and SGI1-C, were tested for their ability to transfer SGI1 to *E. coli*. The transconjugants had the resistance patterns ASSuRif (transconjugant from H16 and *E. coli*) and ASuRif (transconjugant from H18 and *E. coli*) (Table 1). Integrons were detected in these transconjugants.

Discussion

This study describes the antibiotic resistance phenotypes and resistance genes of 26 integron-carrying MDR *Salmonella*

Typhimurium isolates from horses, the ability of these isolates to transfer their antimicrobial resistance determinants to *E. coli*, and their genetic relationship. These data indicate that equine *Salmonella* Typhimurium isolates may be potential risk factors for both animal and human health because they can easily spread their resistance determinants and because of the close contact between horses and humans.

An interesting finding in our study was that isolates of different phage types can have the same PFGE profile, carry the same integron type and show a similar resistance phenotype. Vice versa, isolates of the same phage type can have different PFGE profiles, contain distinct integrons in various genomic islands (SGI1, SGI1-C or SGI1-B) and have different resistance phenotypes. The combination of phage typing, PFGE analysis and the analysis of the integrons indicated that the equine integron-carrying *Salmonella* Typhimurium isolates are not clonal but belong to a number of different strains. These data and the great potential of horizontal transfer indicate that the multidrug resistance is due to acquired resistance rather than to the spreading a single clone.

Apart from resistance to sulphonamides, resistance to ampicillin, chloramphenicol, streptomycin and tetracycline was commonly observed regardless of the phage types of the isolates. Phenotypic resistance to these antimicrobials in the *Salmonella* Typhimurium DT104 isolates may be caused by the presence of the resistance genes [*aadA2*, bla_{PSE-1}, *floR*, *tet*(G) and *sul1*] associated with SGI1. In isolates of phage types other than DT104, integron-associated resistance genes (*aadA1*, *dfrA1*, *dfrA7*, *dfrA14*) were responsible for part of the resistance phenotype detected. In these non-DT104 isolates resistance to gentamicin, kanamycin and enrofloxacin was frequently observed, but integron-associated gene cassettes encoding these resistances were not found. The trimethoprim resistance gene cassettes (*dfrA1*, *dfrA7* and *dfrA14*) were frequently detected in integrons in the present study. This is in accordance with a previous report on high percentages of phenotypic resistance to sulphonamides and trimethoprim in equine salmonellae in The Netherlands.[2] It seems that resistance to trimethoprim and sulphonamides is due to the frequent use of these antimicrobials for the treatment of horses. In The Netherlands, trimethoprim/sulphonamide combinations are the first choice in the treatment of equine salmonellosis. All horses in the present study were clinically ill and were probably treated with trimethoprim/sulphonamides or other antimicrobials before the samples for culturing were taken. However, the exact data on the usage of antimicrobials in the horses were not available.

An important finding was that the nine MDR *Salmonella* Typhimurium isolates carrying a rare integron type with the *dfrA14* and *aadA1* gene cassettes, belong to distinct strains because different phage types and two distinct PFGE patterns were observed. Four of these isolates were able to transfer their integron and the resistance determinants encoding for ampicillin, chloramphenicol and tetracycline resistance to *E. coli.* This clearly indicates the potential of these strains for gene transfer to other members of the Enterobacteriaceae. These nine isolates also showed resistance to flumequine and enrofloxacin. This resistance is caused by mutations leading to the amino acid changes Ser-83 → Ala and Asp-87 → Asn in GyrA. In *Salmonella*, a single mutation in *gyrA* can be sufficient to cause high-level resistance to nalidixic acid but additional mutations are required to attain high-level resistance to fluoroquinolones.[25] In previous studies, single amino acid changes at Ser-83 → Phe and Asp-87 → Asn/Gly were most commonly observed.[26–29] The mutations at both codons mentioned above were previously detected in *in vitro* experiments,[21,30] and they were also described in six *Salmonella* Typhimurium isolates obtained from humans and cattle in Germany.[31] The presence of enrofloxacin-resistant *Salmonella* isolates in Dutch horses is unexpected because quinolones are not licensed for use in horses in The Netherlands. A possible explanation is that these isolates originate from other animal species or humans.

A 90 kb plasmid can be transferred from *Salmonella* Typhimurium to *E. coli*, including the antimicrobial resistance genes that are present on it. In most transconjugants at least one plasmid was present, but in some cases, no plasmid could be observed although the transconjugants had obtained a resistance phenotype similar to that of the donor. A possible explanation for this phenomenon is the presence of a low-copy plasmid, which could not be detected with the procedure used. Another explanation may be that the resistance determinants were present on a conjugative transposon and may be integrated into the chromosome of the recipients.[32]

Another interesting feature of the present study is the presence of a conjugative plasmid-associated integron and a chromosomally located integron in the same *Salmonella* Typhimurium DT104 strain. Evidence for the presence of a conjugative plasmid-associated integron includes the presence of integrons in the *E. coli* transconjugants obtained after mating between *Salmonella* Typhimurium H16 or H18 and *E. coli* K12; and the phenotypic resistance to ampicillin, streptomycin, sulphonamide and rifampicin observed in the transconjugants. *Salmonella* isolates H16 and H18 contained SGI1 and SGI1-C. However, these genomic islands appear not to be transferred because neither resistance to chloramphenicol or tetracycline nor structures of SGI1 in the *E. coli* chromosome were detected in the transconjugants.

A novel variant of SGI1 was found in a *Salmonella* Typhimurium DT104 isolate. In this isolate the *aadA2* gene present in the first integron of SGI1 or its variants (SGI1-A, -C, -D, E, and I)[9,10,19] is replaced by the *aadB* gene. The presence of this new type of SGI1 with the resistance gene cluster *aadB-sul1-floR-tet*(G)-bla_{PSE-1} coincided with the resistance to aminoglycosides, sulphonamides, tetracycline, chloramphenicol/florfenicol and ampicillin observed in this isolate. It is proposed to name this variant SGI1-M. The resistance to streptomycin and trimethoprim is probably not encoded by gene cassettes integrated in integrons in this isolate.

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Transparency declarations

None to declare.

References

1. Su LH, Chiu CH, Chu C *et al*. Antimicrobial resistance in non-typhoid *Salmonella* serotypes: a global challenge. *Clin Infect Dis* 2004; **39**: 546–51.

2. van Duijkeren E, Wannet WJB, Heck MEOC *et al*. Serotypes, phage types and antibiotic susceptibilities of *Salmonella* strains isolated from horses in The Netherlands from 1993 to 2000. *Vet Microbiol* 2002; **86**: 203–12.

3. Espie E, De Valk H, Vaillant V *et al*. An outbreak of multidrug-resistant *Salmonella enterica* serotype Newport infections linked to the consumption of imported horse meat in France. *Epidemiol Infect* 2005; **133**: 373–6.

4. Leverstein-van Hall MA, Blok HEM, Donders ART *et al*. Multidrug resistance among Enterobacteriaceae is strongly associated with the presence of integrons and is independent of species or isolate origin. *J Infect Dis* 2003; **187**: 251–9.

5. Hall RM, Stokes HW. Integrons: novel DNA elements which capture genes by site-specific recombination. *Genetica* 1993; **90**: 115–32.

6. Fluit AC. Towards more virulent and antibiotic-resistant *Salmonella*? *FEMS Immunol Med Microbiol* 2005; **43**: 1–11.

7. Mulvey MR, Boyd DA, Olson AB *et al*. The genetics of *Salmonella* genomic island 1. *Microbes Infect* 2006; **8**: 1915–22.

8. Boyd D, Peters GA, Cloeckaert A *et al*. Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona. *J Bacteriol* 2001; **183**: 5725–32.

9. Doublet B, Butaye P, Imberechts H *et al*. *Salmonella* genomic island 1 multidrug resistance gene clusters in *Salmonella enterica* serovar Agona isolated in Belgium in 1992 to 2002. *Antimicrob Agents Chemother* 2004; **48**: 2510–17.

10. Levings RS, Lightfoot D, Partridge SR *et al*. The genomic island SGI1, containing the multiple antibiotic resistance region of *Salmonella enterica* serovar Typhimurium DT104 or variants of it, is widely distributed in other *S. enterica* serovars. *J Bacteriol* 2005; **187**: 4401–9.

11. Doublet B, Boyd D, Mulvey MR *et al*. The *Salmonella* genomic island 1 is an integrative mobilizable element. *Mol Microbiol* 2005; **55**: 1911–24.

12. van Duijkeren E, Box ATA, Schellen P *et al*. Class 1 integrons in Enterobacteriaceae isolated from clinical infections of horses and dogs in The Netherlands. *Microb Drug Resist* 2005; **11**: 383–6.

13. Leverstein-Van Hall MA, Paauw A, Box ATA *et al*. Presence of integron-associated resistance in the community is widespread and contributes to multidrug resistance in the hospital. *J Clin Microbiol* 2002; **40**: 3038–40.

14. Guinee PA, van Leeuwen WJ, Pruys DPhage typing of *S*. Typhimurium in The Netherlands. 1. The phage typing system. *Zentralbl Bakteriol* 1974; **226**: 194–200.

15. van Duijkeren E, Wannet WJB, Houwers DJ *et al*. Serotype and phage type distribution of *Salmonella* strains isolated from humans, cattle, pigs, and chickens in The Netherlands from 1984 to 2001. *J Clin Microbiol* 2002; **40**: 3980–5.

16. Commissie Richtlijnen Gevoeligheidsbepalingen (CRG). Interpretatie van gevoeligheidsonderzoek en gevoeligheidscriteria voor antibacteriele middelen in Nederland. *Ned Tijdschr Med Microbiol* 2000; **8**: 79–81.

17. Levesque C, Piche L, Larose C *et al*. PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Agents Chemother* 1995; **39**: 185–91.

18. Vo ATT, van Duijkeren E, Fluit AC *et al*. Antibiotic resistance, integrons and genomic island SGI1 among non-typhoid *Salmonella* serovars in The Netherlands. *Int J Antimicrob Agents* 2006; **28**: 172–9.

19. Doublet B, Lailler R, Meunier D *et al*. Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster in *Salmonella enterica* serovar Albany. *Emerg Infect Dis* 2003; **9**: 585–91.

20. Ebner P, Garner K, Mathew A. Class 1 integrons in various *Salmonella enterica* serovars isolated from animals and identification of genomic island SGI1 in *Salmonella enterica* var. Meleagridis. *J Antimicrob Chemother* 2004; **53**: 1004–9.

21. Giraud E, Brisabois A, Martel JL *et al*. Comparative studies of mutations in animal isolates and experimental in vitro- and in vivo-selected mutants of *Salmonella* spp. suggest a counterselection of highly fluoroquinolone-resistant strains in the field. *Antimicrob Agents Chemother* 1999; **43**: 2131–7.

22. Paauw A, Fluit AC, Verhoef J *et al*. *Enterobacter cloacae* outbreak and emergence of quinolone resistance gene in Dutch hospital. *Emerg Infect Dis* 2006; **12**: 807–12.

23. Vo ATT, van Duijkeren E, Fluit AC *et al*. Class 1 integrons in Dutch *Salmonella enterica* serovar Dublin isolates from clinical cases of bovine salmonellosis. *Vet Microbiol* 2006; **117**: 192–200.

24. Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. *J Bacteriol* 1981; **145**: 1365–73.

25. Ruiz J, Castro D, Goni P *et al*. Analysis of the mechanism of quinolone resistance in nalidixic acid-resistant clinical isolates of *Salmonella* serotype Typhimurium. *J Med Microbiol* 1997; **46**: 623–8.

26. Piddock LJ, Ricci V, McLaren I *et al*. Role of mutation in the *gyrA* and *parC* genes of nalidixic-acid-resistant *Salmonella* serotypes isolated from animals in the United Kingdom. *J Antimicrob Chemother* 1998; **41**: 635–41.

27. Chu C, Su LH, Chu CH *et al*. Resistance to fluoroquinolones linked to *gyrA* and *parC* mutations and overexpression of *acrAB* efflux pump in *Salmonella enterica* serotype Choleraesuis. *Microb Drug Resist* 2005; **11**: 248–53.

28. Liebana E, Clouting C, Cassar CA *et al*. Comparison of *gyrA* mutations, cyclohexane resistance, and the presence of class I integrons in *Salmonella enterica* from farm animals in England and Wales. *J Clin Microbiol* 2002; **40**: 1481–6.

29. Griggs DJ, Gensberg K, Piddock LJ. Mutations in *gyrA* gene of quinolone-resistant *Salmonella* serotypes isolated from humans and animals. *Antimicrob Agents Chemother* 1996; **40**: 1009–13.

30. Miko A, Pries K, Schroeter A *et al*. Molecular mechanisms of resistance in multidrug-resistant serovars of *Salmonella enterica* isolated from foods in Germany. *J Antimicrob Chemother* 2005; **56**: 1025–33.

31. Heisig P, Kratz B, Halle E *et al*. Identification of DNA gyraseA mutations in ciprofloxacin-resistant isolates of *Salmonella Typhimurium* from men and cattle in Germany. *Microb Drug Resist* 1995; **1**: 211–18.

32. Fluit AC, Schmitz FJ. Resistance integrons and super-integrons. *Clin Microbiol Infect* 2004; **10**: 272–88.