International Journal of Antimicrobial Agents Volume 28, Issue 3 , September 2006, Pages 172-179

doi:10.1016/j.ijantimicag.2006.05.027

Antibiotic resistance, integrons and *Salmonella* genomic island 1 among non-typhoidal *Salmonella* serovars in The Netherlands

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Received 1 February 2006; accepted 19 May 2006. Available online 17 August 2006.

Abstract

The objective of this study was to investigate the antimicrobial resistance patterns, integron characteristics and gene cassettes as well as the presence of *Salmonella* genomic island 1 (SGI1) in non-typhoidal *Salmonella* (NTS) isolates from human and animal origin. Epidemiologically unrelated Dutch NTS strains (n = 237) originating from food-producing animals and human cases of salmonellosis were tested for their susceptibility to 15 antimicrobial agents. Resistance to 14 of these antimicrobials, including the third-generation cephalosporins, was detected. Resistance to sulphonamides, ampicillin, tetracycline, streptomycin, trimethoprim and nalidixic acid was common (≥10% of the strains were resistant). Resistance against three or more antimicrobials was observed in 57 isolates. The same 237 strains were studied for the prevalence of class 1 integrons, their gene cassettes and the presence of SGI1. Thirty-six isolates (15.2%) carried class 1 integrons. These integrons had ten distinct profiles based on the size of the integron and restriction fragment length polymorphism analysis. Integrons were detected for the first time in serovars Indiana and Senftenberg. Multidrug resistance was strongly associated with the presence of class 1 integrons in which the *aadA2*, *aadA1*, bla_{PSE-1}, *dfrA1*, *dfrA5*, *dfrA14* or *sat* genes were present, as determined by nucleotide sequence determination. The presence of gene cassettes or combinations of gene cassettes not previously found in integrons in *Salmonella* was observed. SGI1 or its variants (SGI-B, -C and -F) were present in 16 isolates belonging to either serovar Typhimurium, Derby or Albany. Regardless of whether the isolate was of human or animal origin, the same resistance phenotype, integron profile and SGI1 structure could be observed.

Keywords: Antimicrobial resistance; Integrons; *Salmonella*; *Salmonella* genomic island 1

1. Introduction

Salmonellosis is a major zoonotic disease in humans, causing 68% of the outbreaks of food-borne diseases reported in Europe between 1993 and 1998. In The Netherlands, 50 000 cases of salmonellosis are reported each year [1]. The majority of the 2500 *Salmonella* serovars can cause food-borne salmonellosis in humans [2].

Antimicrobial resistance in *Salmonella* spp. is a major health problem in human and veterinary medicine worldwide [3]. Many antimicrobial resistance genes are associated with genetic elements called integrons [4], which can be located on transposons and plasmids but also on the chromosome. They are able to integrate and express genes coding for antibiotic resistance [5] and [6]. Class 1 integrons, the most common integron type [5], have been detected in many countries in different *Salmonella* serovars [7] and may be located on the so-called *Salmonella* genomic island 1 (SGI1) [8], [9], [10], [11] and [12]. SGI1 is an integrative 43 kb mobilisable chromosomal element [13] on which antibiotic resistance genes are clustered, flanked by two class 1 integrons [14], [15] and [16]. Strains containing SGI1 are usually resistant to ampicillin (and amoxicillin), streptomycin, spectinomycin, chloramphenicol (and florfenicol), sulphonamides and tetracycline. Strains carrying SGI1 variants (SGI1-A to SGI1-J) with different antibiotic resistance profiles have also been found in several *Salmonella* serovars [9], [11], [12] and [17]. It is important to study the spread of antibiotic resistance to understand the relationship between antibiotic resistance genes, class 1 integrons and SGI1, as integrons and transferable elements are responsible for today's spread of resistance genes in the bacterial population and increase the overall resistance gene pool.

The aim of the present study was to determine (1) the antimicrobial resistance profiles of *Salmonellae* isolated from humans, cattle, pigs, chickens and food products in The Netherlands, (2) the prevalence and molecular characteristics of class 1 integrons in these strains and (3) the genetic basis of SGI1.

2. Materials and methods

2.1. Bacteria

The 237 epidemiologically unrelated *Salmonella* isolates in this study were derived from a collection of 3265 isolates obtained in 2004 by the Dutch National Institute of Public Health and the Environment (RIVM) and the Veterinary Microbiological Diagnostic Center of Utrecht University. All isolates were confirmed to be *Salmonella* based on colony morphology and biochemical tests [18]. The isolates in this study were chosen to represent different serotypes and different origins of isolation, i.e. humans (n = 114), cattle (n = 44), pigs (n = 24), chickens (n = 47) and food products (meat or eggs; n = 8). All human isolates were from clinical cases. Of the animal isolates, 73% were from clinical cases (mainly cattle and pigs) and 17% were from healthy animals (mostly chickens). The isolates were serotyped according to the latest version of the Kauffmann–White scheme using slide and microtitre plate agglutination [19]. Serovar Enteritidis isolates were phage-typed as described by Ward et al. [20]. Serovar Typhimurium isolates were phage-typed using the Dutch phage typing system [21].

2.2. Antimicrobial susceptibility

Antimicrobial susceptibility was determined by the agar diffusion technique using commercial disks (Oxoid Ltd., Basingstoke, UK) according to the guidelines of the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) [22]. The antimicrobials tested were: ampicillin 10 μg, amoxicillin/clavulanic acid 30 μg, cefalothin 30 μg, ceftazidime 30 μg, chloramphenicol 30 μg, ciprofloxacin 5 μg, colistin 10 μg, gentamicin 10 μg, kanamycin 30 μg, nalidixic acid 30 μg, norfloxacin 10 μg, streptomycin 10 μg, tetracycline 30 μg, trimethoprim 5 μg and sulphonamides 300 μg. *Escherichia coli* 25922 was used as the control organism.

2.3. Detection of class 1 integrons

Template DNA of all 237 isolates was prepared by the boiled lysate procedure [23]. Integrons were detected by polymerase chain reaction (PCR) amplification of the class 1 integrase-specific *int1* gene [24]. Subsequently, the size of any inserted gene cassette of the integrase-positive isolates was determined by PCR using primers for the conserved segment regions (CS-PCR) [23]. Since the 3′ segment of the class 1 integron is not always conserved, some integrase-positive isolates yielded no product in the CS-PCR. In this case, the gene cassette of the isolate was characterised by an inverted PCR for the integrase gene. Briefly, 1 μg of genomic DNA was cleaved with the restriction endonuclease *Sph*I. The DNA obtained in this way was ligated and subjected to PCR using the int-OUT and CS-F primers (Table 1). Only amplicons of ca. 1 kb were sequenced. CS-PCR products were resolved by electrophoresis at 100 V for 3 h on 0.7% agarose gels containing ethidium bromide and visualised under ultraviolet light.

Table 1.
Primers used for polymerase chain reaction

Primer	Gene	Fragment amplification	Product size	Sequence (5′–3′)	Reference
int1-F	*int1*	*int1*	242	TCT CGG GTA ACA TCA AGG	Leverstein et al., 2002 [24]
int1-R	*int1*			AGG AGA TCC GAA GAC CTC	Leverstein et al., 2002 [24]
int-OUT	*int1*	Cassette(s)	[a]	AAG TGG TTC GCA TCC TCG	This study
CS-F	Conserved segment	Cassette(s)	[a]	GGC ATC CAA GCA GCA AG	Levesque et al., 1995 [23]
CS-R	Conserved segment			AAG CAG ACT TGA CCT GA	Levesque et al., 1995 [23]
U7-L12	*thdf*	Left junction	500	ACA CCT TGA GCA GGG CAA G	Doublet et al., 2003 [12]
LJ-R1	*int1*			AGT TCT AAA GGT TCG TAG TCG	Doublet et al., 2003 [12]
104-RJ	S044	Right junction	515	TGA CGA GCT GAA GCG AAT TG	Doublet et al., 2003 [12]
C9-L2	*int2*			AGC AAG TGT GCG TAA TTT GG	Doublet et al., 2003 [12]
104-D	*yidY*		500	ACC AGG GCA AAA CTA CAC AG	Doublet et al., 2003 [12]
dfrA1-F	*dfrA1*	*dfrA1..orfC*	1057	CCA GCA GCA AGC GCG TTA CG	This study
orfC-R	*orfC*			TCT CGA ATC AAG CAG GAA CC	This study
cml01	*floR*	*floR*	494	TTT GGW CCG CTM TCR GAC	Doublet et al., 2003 [12]
cml15	*floR*			SGA GAA RAA GAC GAA GAA G	Doublet et al., 2003 [12]
int1	*intI1*	*intI..aadA2*	1135	GCT CTC GGG TAA CAT CAA GG	Doublet et al., 2003 [12]
aad	*aadA2*			GAC CTA CCA AGG CAA CGC TA	Doublet et al., 2003 [12]
sulTER	*sul1delta*	*sulI..floR*	942	AAG GAT TTC CTG ACC CTG	Doublet et al., 2003 [12]
F3	*floR*			AAA GGA GCC ATC AGC AGC AG	Doublet et al., 2003 [12]

Primer	Gene	Fragment amplification	Product size	Sequence (5′–3′)	Reference
F4	*floR*	*floR..tetR*	598	TTC CTC ACC TTC ATC CTA CC	Doublet et al., 2003 [12]
F6	*tetR*			TTG GAA CAG ACG GCA TGG	Doublet et al., 2003 [12]
tetR	*tetR*	*tetR..tetA*	1559	GCC GTC CCG ATA AGA GAG CA	Doublet et al., 2003 [12]
tetA	*tetA*			GAA GTT GCG AAT GGT CTG CG	Doublet et al., 2003 [12]
int2	*groEL-intI1*	*int1..pse1*	1338	TTC TGG TCT TCG TTG ATG CC	Doublet et al., 2003 [12]
pse1	*pse-1*			CAT CAT TTC GCT CTG CCA TT	Doublet et al., 2003 [12]
pse-L	*pse-1*	*pse1..S044*	4400	AAT GGC AAT CAG CGC TTC CC	Doublet et al., 2003 [12]
MDR-B	S044			GAA TCC GAC AGC CAA CGT TCC	Doublet et al., 2003 [12]
TEM-F	bla_{TEM}	bla_{TEM}		ATG AGT ATT CAA CAT TTC CGT GTC G	This study
TEM-R	bla_{TEM}			ACC AAT GCT TAA TCA GTG AGG CA	This study
TEM-S1	bla_{TEM}	For sequencing		ACA ACG ATC GGA GGA CCG	This study
TEM-S2	bla_{TEM}	For sequencing		GCG GTT AGC TCC TTC GGT	This study
SHV-F	bla_{SHV}	bla_{SHV}		GTA TTG AAT TCA TGC GTT ATA TTC GCC TGT GTA	Bradford, 1999 [25]
SHV-R	bla_{SHV}			CAG AAT TCG GCT AGC GTT GCC AGT GCT CGA	Bradford, 1999 [25]
CTX-F	bla_{CTX-M}	bla_{CTX-M}	538	CGA TGT GCA GTA CCA GTAA	A. Paauw, 2006[b]
CTX-R	bla_{CTX-M}			ATA TCG TTG GTG GTG CC	A. Paauw, 2006[b]

[a] Size depending on the gene cassette(s) inserted.
[b] Personal communication.

2.4. Gene cassette characterisation

CS-PCR amplicons of the same size were restricted with two restriction endonucleases and were considered identical if they had the same restriction fragment length polymorphism (RFLP) pattern after digestion with both enzymes (Table 2). One sample of each representative RFLP type was randomly chosen for sequencing. In the case of a unique integron, purified CS-PCR products were cloned in the pGEM-T easy Vector (Promega, Madison, WI). Colonies containing plasmids with the inserted fragment were picked from Luria–Bertani plates containing ampicillin (100 μg/mL), 40 μL of IPTG (100 mM per plate) and 40 μL of X-Gal (2% per plate). The target fragments were obtained by PCR using T7 and Sp6 primers under the same conditions as described for the CS-PCR. The amplification products were purified using the Qia Gel Extraction kit (Qiagen, Hilden, Germany) and the nucleotide sequence was determined. For sequencing of the different amplicons from both ends, T7 and Sp6 primers were used. For the CS amplicon of 2000 bp, an internal primer was synthesised (based on the sequence obtained) and used to continue sequencing until the resistance genes inserted in the amplicon were identified. For isolates containing two integrons that only differed by 50 bp in size, CS-PCR products were cloned in the pGEM-T easy Vector (Promega). Plasmids with different inserts were selected on the basis of restriction enzyme analysis using *Eco*RI or *Hpa*II. Purified plasmids were then used for DNA sequence determination. Dideoxy sequencing was performed on an ABI 3730 Sequencer (Applied Biosystems, Foster City, CA). DNA sequences were analysed with the Clone Manager Suit and by consulting the GenBank database of the National Center for Biotechnology Information via the BLAST network service. The nucleotide sequences of the gene cassettes have been deposited in the GenBank database under the accession numbers given in Table 2.

Table 2.

Characterisation of class 1 integrons of *Salmonella* isolates in The Netherlands

IP	Size in bp (isolate ID)	RE 1	Fragments (ca. bp)	RE 2	Fragments (ca. bp)	Gene cassette	Accession number
I	1000 (N216)	*Eco*RI	550, 450	*Hpa*II	400, 250, 150, 50	*aadA2*	DQ133165
	1200	*Hinc*II	700, 350, 150	*Hpa*II	800, 400	bla_{PSE-1}	DQ133161
II	1250 (N107)					*dfrA1*	DQ123842
	1200					bla_{PSE-1}	DQ133163
III	700 (N111)					*dfrA5*	DQ133160
IV	1000 (N328)	*Bcl*I	700, 300	*Hpa*II	580, 240, 120, 60	*aadA1*	DQ133159
V	1000 (N80)	*Eco*RI	550, 450	*Hpa*II	400, 250, 150, 50	*aadA2*	DQ133165
VI	1200 (N209)	*Hinc*II	700, 350, 150	*Hpa*II	800, 400	bla_{PSE-1}	DQ133162
VII	1500 (N133)	*Hinc*II	1000, 500	*Hpa*II	700, 500, 200, 100	*aadA1*, *dfrA1*	DQ133164
VIII	2000 (N279)	*Bcl*I	700, 650, 450	*Hpa*II	700, 500, 300, 200, 100	*aadA2*, *sat*	DQ133166
IX	1200 (N246)					*dfrA14*, *orfC*	DQ228133
X	— (N178)[a]						

IP, integron profile; RE, restriction endonuclease.
[a] No product found from conserved segment polymerase chain reaction (CS-PCR) or inverted PCR.

2.5. Salmonella genomic island 1 mapping

Integron-positive isolates were investigated for the presence of SGI1. The isolates were first examined by PCR for the presence of the left and right junction of SGI1. Next, the presence of sequences from the antibiotic resistance gene cluster was determined by PCR using primers (Table 1) described previously [12] and in this study. Template DNA was prepared with the High Pure PCR Preparation Kit (Roche, Mannheim, Germany). PCR was performed in a total volume of 25 μL containing 2.5 μL of 10× PCR buffer (HT Biotechnology, Cambridge, UK), 0.5 μL of 10× deoxynucleotide triphosphate mix (2 mM each), 50 pmol of each primer, 1.25 U of *Taq* DNA polymerase and 1 μL of template DNA. To amplify fragments larger than 3.5 kb, *Taq* Plus polymerase was used instead of *Taq* DNA polymerase (HT Biotechnology). Thermal cycling conditions consisted of a hot start cycle at 94 °C for 3 min, followed by 35 cycles of 1 min at 94 °C, 1 min at 50–65 °C (depending on the primers used), 1–5 min at 72 °C (depending on the expected amplicon size) and a final step of 72 °C for 10 min. Annealing temperatures for primers used in the SGI1 mapping were 50 °C except for int1/aad, tetR/tetA (54 °C), sulTER/F3 and pse-L/MDR-B (60 °C).

2.6. β-Lactamase production

Ceftazidime-resistant isolates were investigated for extended-spectrum β-lactamase (ESBL) production. The isolates were also tested for TEM, SHV and CTX-M type β-lactamases by PCR amplification using the primers listed in Table 1 [25]. The expected product was sequenced and analysed according to Lahey's scheme [26]. *Escherichia coli* 09A488, *Klebsiella pneumoniae* 09A018 and *Enterobacter cloacae* 03773, which were previously shown to harbour TEM, SHV and CTX-M type β-lactamase, respectively, were used as positive controls.

2.7. Statistical analysis

All statistical analyses were performed in Microsoft Excel 2000 using χ^2 tests. Differences were considered significant at $P < 0.05$.

3. Results

In the present study, 128 isolates (54%) were susceptible to all 15 antibiotics tested (Fig. 1). Sixty-nine *Salmonella* (29.1%) were resistant to two or more antimicrobials. Antimicrobial resistance was not solely associated with a particular *Salmonella* serovar. Resistant isolates belonged to 25 different serovars, including Typhimurium (35/51), Dublin (10/37), Enteritidis (13/41), Virchow (8/8), Hadar, Paratyphi B (5/5), Derby (6/7), Newport (3/6), Mbandaka (3/7), Brandenburg (2/6), Senftenberg (2/7), Stanley (2/3), Livingstone (1/2), Agona (1/3), Anatum (1/3), Panama (1/4), Infantis (1/5), Albany, Blockley, Braenderup, Corvallis, Haifa, Glostrup, Indiana and London (1/1). However, resistance to antimicrobials was high (nearly 70%) in serovar Typhimurium. *Salmonella* Typhimurium PT 506 (DT 104) isolates were resistant to between two and seven antimicrobials. Resistance to sulphonamides, ampicillin, tetracycline, streptomycin, trimethoprim, nalidixic acid, chloramphenicol, cefalothin, amoxicillin/clavulanic acid, ceftazidime, kanamycin, ciprofloxacin, norfloxacin and gentamicin was found in 29%, 21%, 17%, 17%, 13%, 12%, 9%, 4%, 2%, 1%, 0.8%, 0.8%, 0.4% and 0.4% of the isolates, respectively.

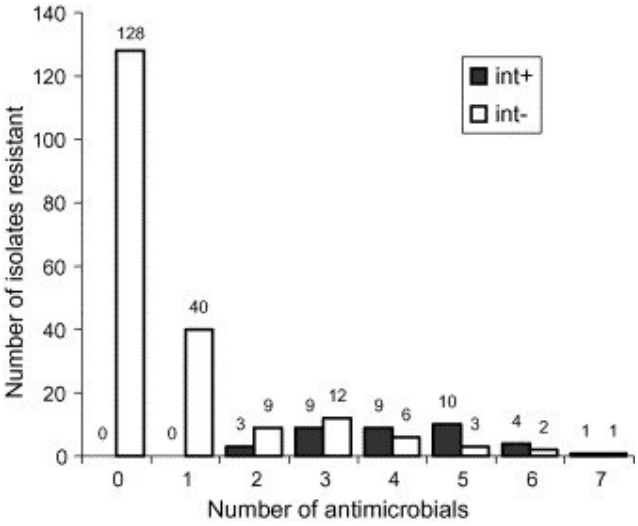

Fig. 1. Relationship between class 1 integrons and multidrug resistance (χ^2 test, $P < 0.001$).

Most isolates in the present study were susceptible to the third-generation cephalosporins. However, three (1.3%) ceftazidime-resistant isolates (two *S*. Typhimurium from human and cattle and one *S*. Braenderup from chicken) were observed. No ESBL-producing strain was found.

Resistance to nalidixic acid was common in serovar Hadar (100%) and serovar Virchow (87.5%). Three isolates showing resistance to fluoroquinolones (norfloxacin (1) and ciprofloxacin (2)) and three isolates with decreased susceptibility (reduction in diameter of the inhibition zone) to these antimicrobials (norfloxacin (2) and ciprofloxacin (1)) were present. Multidrug-resistant *Salmonella* were obtained more frequently from animal origin (86%, 50%, 30% and 23% of food, pig, cattle and chicken isolates, respectively) than from humans (19%).

There was a strong relationship between multidrug resistance and the presence of class 1 integrons ($P < 0.001$) (Fig. 1). Class 1 integrons were detected in 36 *Salmonella* isolates (15.2%) of 11 different serovars. Integrons were found predominantly in *S*. Typhimurium DT 104 ($n = 13$; 100%). However, class 1 integrons were also identified in other phage types of *S*. Typhimurium and in other serovars (Table 3). Data analysis showed that there is a strong relationship between the presence of class 1 integrons and resistance to sulphonamides, chloramphenicol, ampicillin, tetracycline, streptomycin or trimethoprim ($P < 0.001$ in order of decreasing strength of correlation), whilst resistance to other antimicrobials tested was integron independent.

Table 3.

Resistance patterns in class 1 integron-positive *Salmonella enterica* isolates, their integrons and *Salmonella* genomic island (SGI1) types

ID	Resistance profile (intermediate)	Serovar (source of isolate[a])	Amplicon size (bp)	IP[b]	SGI types
N216	AMP, CAZ, CHL, STR, TET, TMP, SUF (AMC)	Typhimurium DT 104[c] (H)	1000; 1200	I	SGI1
N235	AMP, CHL, STR, TET, TMP, SUF (AMC)	Typhimurium DT 104 (P)	1000; 1200	I	SGI1
N246	AMP, CHL, STR, TET, TMP, SUF (AMC)	Senftenberg (C)	1200[d]	IX	—
N193	AMP, CHL, NAL, TET, TMP, SUF (AMC, STR)	Derby (H)	1000; 1200	I	SGI1
N140	AMP, CEP, NAL, TET, TMP, SUF (AMC, STR)	Virchow (C)	1500	VII	—
N94	AMP, CHL, STR, TET, SUF (AMC)	Typhimurium DT 104 (P)	1000; 1200	I	SGI1
N176	AMP, CHL, STR, TET, SUF (AMC)	Typhimurium DT 104 (H)	1000; 1200	I	SGI1
N182	AMP, CHL, STR, TET, SUF (AMC)	Typhimurium DT 104 (H)	1000; 1200	I	SGI1
N188	AMP, CHL, STR, TET, SUF	Typhimurium DT 104 (H)	1000; 1200	I	SGI1
N221	AMP, CHL, STR, TET, SUF (AMC)	Typhimurium DT 104 (H)	1000; 1200	I	SGI1
N326	AMP, CHL, STR, TET, SUF (AMC)	Non-DT 104[e] (B)	1000; 1200	I	SGI1
N104	AMP, CHL, NAL, STR, SUF (TET)	Typhimurium DT 104 (M)	1000; 1200	I	SGI1
N167	AMP, KAN, TET, TMP, SUF (CEP)	Non-DT 104 (M)	2000	VIII	—
N203	AMP, STR, TET, TMP, SUF	Indiana (C)	1500	VII	—

ID	Resistance profile (intermediate)	Serovar (source of isolate[a])	Amplicon size (bp)	IP[b]	SGI types
N107	AMP, CHL, NAL, TMP, SUF (AMC, STR, TET)	Albany (C)	1200; 1250	II	SGI1-F
N108	AMP, CHL, STR, SUF (AMC, TET)	Typhimurium DT 104 (H)	1000; 1200	I	SGI1
N320	AMP, CHL, STR, SUF (AMC, TET)	Typhimurium DT 104 (B)	1000; 1200	I	SGI1
N194	AMP, CHL, STR, SUF (AMC, TMP)	Non-DT 104 (H)	1000; 1200	I	SGI1
N209	AMP, AMC, NAL, SUF	Typhimurium DT 104 (H)	1200	VI	SGI1-B
N327	CHL, NOR, TMP, SUF	Typhimurium DT 104 (B)	1000	V	—
N279	AMP, STR, TET, SUF (AMC, CEP)	Non-DT 104 (M)	2000	VIII	—
N133	AMP, TET, TMP, SUF (STR)	Virchow (H)	1500	VII	—
N149	AMP, TET, TMP, SUF	Infantis (C)	—	X	—
N111	NAL, TET, TMP, SUF	Haifa (H)	700	III	—
N295	CHL, STR, SUF	Dublin (B)	1000	IV	—
N302	CHL, STR, SUF	Dublin (B)	1000	IV	—
N305	CHL, STR, SUF	Dublin (B)	1000	IV	—
N308	CHL, STR, SUF	Dublin (B)	1000	IV	—
N311	CHL, STR, SUF	Dublin (B)	1000	IV	—
N323	CHL, STR, SUF	Dublin (B)	1000	IV	—
N328	CHL, STR, SUF	Dublin (B)	1000	IV	—

ID	Resistance profile (intermediate)	Serovar (source of isolate[a])	Amplicon size (bp)	IP[b]	SGI types
N199	AMP, TMP, SUF	Mbandaka (C)	—	X	—
N277	TET, TMP, SUF (STR)	Non-DT 104 (P)	1500	VII	—
N80	STR, SUF	Typhimurium DT 104 (P)	1000	V	SGI1-C
N74	STR, SUF (CIP)	Derby (P)	2000	VIII	—
N178	TMP, SUF (STR)	Livingstone (H)	—	X	—

AMP, ampicillin; CAZ, ceftazidime; CHL, chloramphenicol; STR, streptomycin; TET, tetracycline; TMP, trimethoprim; SUF, sulphonamides; AMC, amoxicillin/clavulanic acid; NAL, nalidixic acid; CEP, cefalothin; KAN, kanamycin; NOR, norfloxacin; CIP, ciprofloxacin; —, not found.

[a] H, human; P, pig; C, chicken; B, bovine; M, meat.

[b] Class 1 integron profiles.

[c] DT 104: pt 506 in Dutch phage typing system.

[d] Amplicon of inverted-integrase polymerase chain reaction.

[e] Non-DT 104: *S*. Typhimurium other phage types than pt 506.

Ten class 1 integron profiles (IPs) could be defined based on the number and size of the amplicons obtained (Table 2 and Table 3). Four isolates (of serovars Infantis, Livingstone, Mbandaka and Senftenberg) were integron-positive but yielded no product in the CS-PCR. IP IX was found in *S*. Senftenberg using inverted PCR. IP X was observed in three isolates (*S*. Infantis, *S*. Livingstone and *S*. Mbandaka) where no products in either CS-PCR or inverted PCR were obtained. Gene cassettes encoding resistance to streptomycin (*aadA1*), streptomycin and spectinomycin (*aadA2*), streptothricin (*sat*), β-lactam (bla_{PSE-1}) and trimethoprim (*dfrA1*, *dfrA5* and *dfrA14*) were observed.

Complete copies of SGI1 or one of its variants were found in 16 (44%) of the class 1 integron-positive isolates (Fig. 2; Table 3). SGI1 was found in 12 serovar Typhimurium and one *S*. Derby isolate. SGI1-F was present in a single *S*. Albany isolate. There was a strong relationship between the presence of two class 1 integrons and the presence of SGI1 ($P < 0.001$). Two other isolates of *S*. Typhimurium carrying only a single integron contained an incomplete SGI1, known as SGI1-B (strain N209) or SGI1-C (strain N80). Both yielded the expected products for the left (*thdf* and *int* genes) and right (S044 and *int2* genes) junction of these SGI1s. Isolate N80 also yielded a single 1200 bp product in the amplification of the fragment between the *int1* and *aadA2* genes. Isolate N209 yielded no products in the int2-pseL PCR, but a 900 bp product was generated by amplification with the int1 and pseL primers. No products were obtained from other combined PCRs, as shown in Fig. 2, except for amplification of the 4.5kb fragment from *pse-1* to S044 for isolate N209. Thus, the *Salmonella* genomic islands in strains N209 and N80 (Table 2) were classified as SGI1-B and -C, respectively.

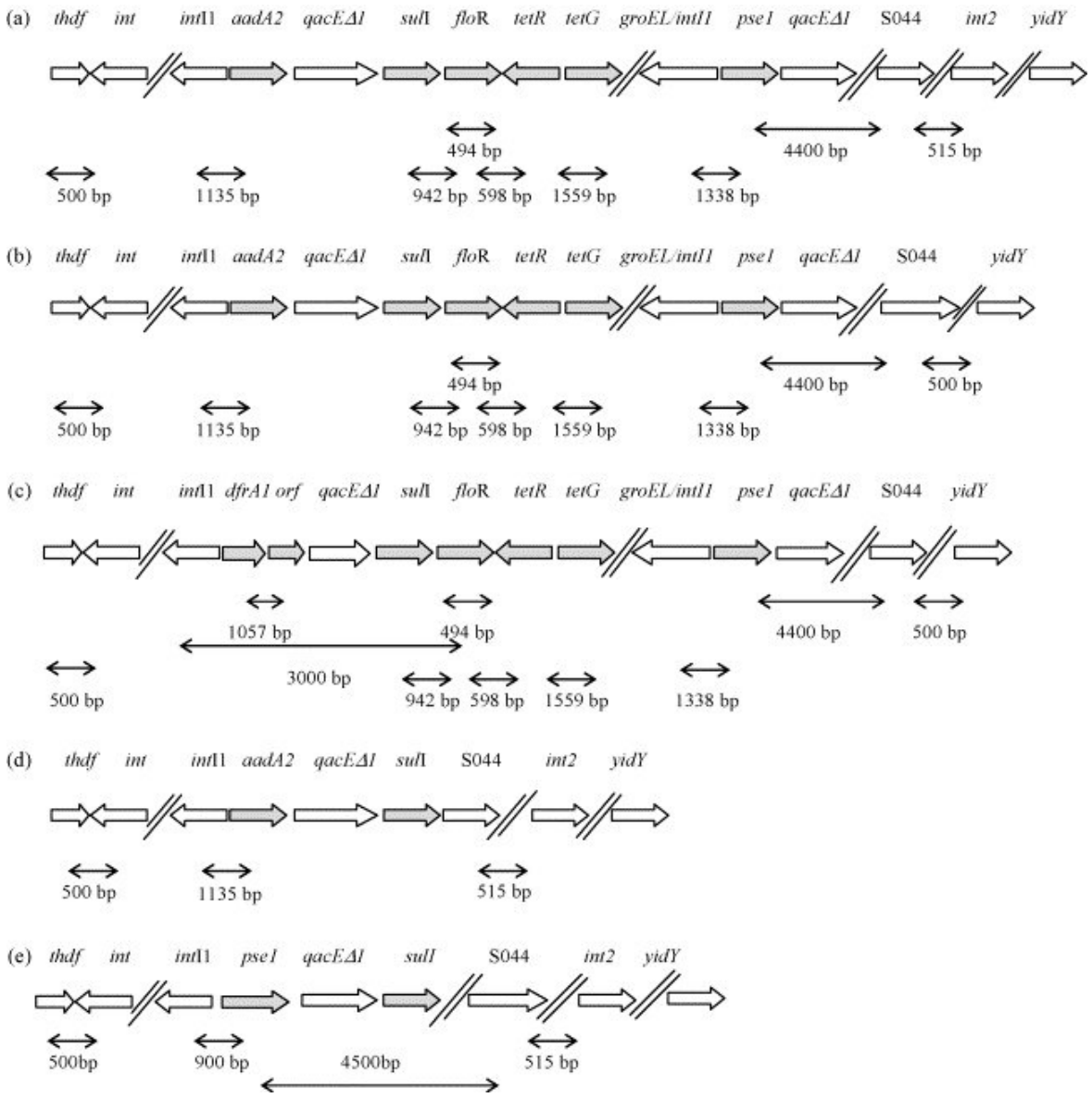

Fig. 2. Map of *Salmonella* genomic island 1 (SGI1) highlighting targets and fragment sizes in polymerase chain reactions used for mapping SGI1 antibiotic resistance gene clusters. (a) SGI1 found in *S*. Typhimurium (N216); (b) SGI1 found in *S*. Derby (N193); (c) SGI1-F found in *S*. Albany (N107); (d) SGI1-C found in *S*. Typhimurium (N80); and (e) SGI1-B found in *S*. Typhimurium (N209). The grey shades indicate resistance genes (modified from [12]).

4. Discussion

The main finding in this study was the widespread presence of multidrug resistance among *Salmonella* from human and animal origin and the diversity of class 1 integrons and SGI1 in different *Salmonella* serovars, including serovars in which class 1 integrons have not previously been found. Class 1 integrons have previously been detected in different *Salmonella* serovars including Typhimurium, Virchow, Hadar, Dublin, Derby, Haifa, Infantis, Albany and Mbandaka [27], [28] and [29]; however, to our knowledge, they have not been found in *S*. Senftenberg and *S*. Indiana. This finding suggests the spread of integrons that

are located on a mobile genetic element among the many serovars of *Salmonella*. In contrast to a study from Scotland [30], no class 1 integrons were found among serovar Enteritidis in this study. In The Netherlands, antimicrobial resistance in *S*. Enteritidis isolates is still low (3%) [31] and [32], whereas in Scotland *S*. Enteritidis isolates were multiresistant and carried integrons [30]. In general, our data suggest that acquisition of integrons is not limited to specific *Salmonella* serovars but may occur in any serovar. Amplification of gene cassettes by CS-PCR in integrase-positive isolates is sometimes negative because the primers do not always anneal to the 3'-conserved segment [23]. This was also observed in the present study. An inverted-integrase PCR proved to be an effective method for the study of (at least part of) the gene cassettes present therein.

Most integron-positive serovars carried gene cassettes that have been described previously [27], [28], [33], [34] and [35]. However, neither the *dfrA5* gene cassette in *S*. Haifa nor the combination of the gene cassettes *aadA2* and *sat* in *S*. Typhimurium or *dfrA14* and an unknown gene in *S*. Senftenberg have been reported before. The *dfrA5* gene has been found in *S*. Wien (accession number AY827837). The *aadA2* and *sat* genes have been described on a plasmid of a highly invasive and resistant *S*. Choleraesuis strain [36]. The gene *dfrA14* has only previously been observed in *E. coli* (accession number AJ884725) but not in *Salmonella*. Therefore, one may speculate that integrons or the gene cassettes they contain have been exchanged not only among *Salmonella* serovars but also among Enterobacteriaceae. Two class 1 integrons with sizes of ca. 1000 bp and 1200 bp were found in almost all *S*. Typhimurium DT 104 isolates, in accordance with the findings of Randall et al. [28]. In the present study, *S*. Typhimurium DT 104 isolates not only had a pentadrug resistance phenotype (ACSSuT), a tetradrug resistance phenotype (CSSuT) or a SSu resistance phenotype, as reported previously [27], [28], [29] and [37], but also other multiple resistance phenotypes (Table 3). These phenotypes are characteristic of SGI1 and its variants.

SGI1 is an important determinant of multidrug resistance. SGI1 or its variants with different gene cassettes located in two integrons have been found in many serovars of *Salmonella enterica* [11]. The high rate with which SGI1 is present in S. Typhimurium DT 104 isolates in the present and previous studies [10], [11] and [12] suggests that the SGI1 variant with the five antibiotic resistance genes *aadA2*, *sul1*, *floR*, *tet* and *pse* is the oldest and probably the first to arise. Its variants in other *Salmonella* serovars [11] probably arose as the result of recombination events. Deletion is a possible explanation for the structure of SGI1-B and SGI1-C in the two *S*. Typhimurium isolates that contain only one integron flanked by the left and right junction of SGI1. The fact that PCR products for isolate N209 could only be obtained with int1-pseL primers but not with int2-pseL primers suggests another location for the bla_{PSE-1} gene at the left junction of SGI1 owing to a single crossover event at *intI1* [9]. The genes found adjacent to the left junction of SGI1, such as the *int* and *xis* genes, could have an important role in the excision and integration of integron-located gene cassettes [14]. In this study, the expression of the tetracycline resistance gene (*tetG*) varied from full resistance among SGI1-carrying *S*. Typhimurium isolates to intermediate susceptibility in an SGI1-F-positive *S*. Albany isolate. Since a promoter is not included in the cassette, the level of antibiotic resistance expressed by the cassette gene is influenced by the position of the cassette in the array if more than one cassette is present. In all cases the resistance level was highest when the gene was closest to the promoter [38], which is located in the 5'-conserved segment of the class 1 integron [5].

In summary, multidrug resistance was strongly associated with the presence of class 1 integrons in *Salmonella*. We found gene cassettes or combinations of gene cassettes not previously reported in integrons in *Salmonella*. Class 1 integrons were detected for the first time in serovars Indiana and Senftenberg. SGI1 and its variants (SGI1-B, -C and -F) were found in serovars Typhimurium, Derby and Albany. Similar resistance patterns, integron types and SGI1 structures were found regardless of the source of the isolate (human or animal).

Acknowledgments

This study was funded by a grant of the Vietnamese government. We thank Max Heck and Frans Bensink of RIVM; Marcel de Zoete of I&I for technical help; and Armand Paauw of Eijkman-Winkler Institute for providing control strains.

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