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Mechanisms

Real-Time PCRs and Fingerprinting Assays for the Detection and Characterization of *Salmonella* Genomic Island-1 Encoding Multidrug Resistance: Application to 445 European Isolates of *Salmonella*, *Escherichia coli*, *Shigella*, and *Proteus*

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Salmonella Genomic Island-1 (SGI-1) harbors a cluster of genes encoding multidrug resistance (MDR). SGI-1 is horizontally transmissible and is therefore of significant public health concern. This study presents two novel real-time PCRs detecting three SGI-1 protein-coding genes and a SGI-1 fingerprinting assay. These assays were applied to 445 European enterobacterial isolates. Results from real-time PCRs were comparable to those obtained from gel-based PCRs used for the detection of SGI-1, but were rapid to perform and suitable for large-scale screening. Furthermore, real-time PCRs also detected SGI-1 even when only part of the island was present in bacterial isolates. No trace of SGI-1 was detected in isolates other than *Salmonella enterica.* The fingerprints showed that regions of SGI-1 outside the MDR region exhibited genomic variations between isolates. In conclusion, the real-time PCRs described here are suitable for the detection of SGI-1 in bacterial isolates. Further studies are necessary to elucidate divergence in its non-MDR region.

Introduction

The increasing prevalence, worldwide, of multidrug-resistant strains of *Salmonella enterica*, serovar Typhimurium definite phage type 104 (*S.* Typhimurium DT104)[15,26,27,31] lead to the identification[3,23–25] and sequencing of a 13 kilobase (kb) chromosomal multidrug resistance (MDR) region in those isolates.[7] The MDR region was found to be located in a 43kb genomic island (*Salmonella* Genomic Island-1 or SGI-1)[5] and the complete sequence of this island was determined soon afterwards.[6] Subsequently, SGI-1 has been detected worldwide in strains of Typhimurium, in other *S. enterica* serovars[2,8,9,13,16,18,21,28,30,32] and in one clinical isolate of *Proteus mirabilis*.[1]

The MDR region has been intensively studied and based on the variability of this region, so far 15 SGI-1 variants (SGI-1, A–N) have been identified and characterized.[4,10,14,19,29] However little is known about the SGI-1 sequences encompassing the MDR region, except for the complete SGI-1 sequence from one single multidrug-resistant Typhimurium DT104 isolate.[5,6]

SGI-1 is a mobilizable element which can be transmitted horizontally amongst *S. enterica* serovars and which inserts stably at a specific site in the bacterial chromosome.[12] The spread of the MDR phenotype amongst the bacterial population is worrying and the detection of SGI-1 is regarded important for public health. SGI-1 is usually identified by showing the presence of SGI-1 left and right junctions by

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published gel-based PCR assays.[5,6,11,17] Primers used in these PCRs are designed to bind on the *Salmonella* chromosome adjoining the left and right junctions of the island. These PCRs could therefore not detect the junctions in the SGI-1 bearing *P. mirabilis* isolate.[1] Furthermore, sequences flanking the insertion point of SGI-1 in *Escherichia coli* differ from those in *Salmonella*.[12] These PCRs may therefore not be suitable to detect SGI-1 in Enterobacteriaceae other than *Salmonella*.

Here we describe novel assays for the detection and characterization of SGI-1. These assays were applied to a wide range of multidrug-resistant *Salmonella* and other Enterobacteriaceae isolates sent by 11 laboratories from nine European countries.

Materials and Methods

Strains

The Health Protection Agency (HPA), Laboratory of Enteric Pathogens (LEP) received 445 strains (277 *S. enterica*, 116 *E. coli*, 43 *Shigella* spp., and 9 *Proteus* spp.) between May 2006 and March 2007, from 11 participating European laboratories. The selection criteria were based on suspicion of multidrug resistance pattern and therefore of harboring SGI-1. The strains had been isolated between 2001 and 2006 from human, food, or animals. Details, when available, of the strain species, serovar, phagetype, resistance to the antimicrobial drugs amoxicillin (A), chloramphenicol (C), streptomycin (S), sulphonamides (Su), and tetracyclines (T) can be seen in Table 1. Resistance to antimicrobials other than those described above was not always communicated by the sending laboratory and is therefore not described further.

Strains used as controls were Typhimurium DT104, strain 96-5227, from which SGI-1 was first sequenced,[5] kindly provided by Dr. Michael Mulvey from the Public Health Agency of Canada, and Typhimurium DT104, strain P5374140, provided by the HPA-LEP (Table 1).

Nucleic acid extraction

One colony of each strain was resuspended in 100 µl of sterile distilled water (Sigma, Gillingham, UK) and heated to 100°C for 10 min. Debris was pelleted by centrifugation at 12,000 *g* for 5 min and the supernatant stored at − 20°C.

PCRs using gel-based detection system

Taq DNA polymerase, the PCR buffer, and $MgCl_2$ were from Bioline, London, UK. Primers were purchased from Invitrogen, Paisley, UK. All PCR assays were performed on a T1 thermocycler from Biometra, Goettingen, Germany. PCR detections of SGI-1 left and right junctions and of class 1 integron gene cassettes were performed using primers previously described: U7L12/LJR1 (SGI-1 left junction); 104RJ/C9L2, or 104D (SGI-1 right junction with or without the retronphage element, respectively); L1/R1 (5′ and 3′ conserved region of class 1 integron).[5,6,11,17] PCR amplifications for detecting the left or right junctions were performed in 25 µl with 5 µl of boiled extracts in 1×PCR buffer, 0.25 mM of each dNTPs, 1.5 mM $MgCl_2$, 0.4 µM of each primers, and 0.375 U of *Taq* DNA polymerase. Samples were subjected to an initial denaturation at 94°C for 1 min, followed by 35 cycles of 94°C for 1 min, 56°C for 30 sec and 72°C for 30 sec followed by a final extension at 72°C for 5 min. PCR amplification for detecting class 1 integron gene cassettes was performed in the same condition, except that the final reaction volume was 50 µl, the $MgCl_2$ concentration was 2.5 mM and the elongation time in each cycle was 2 min.

Amplified products were detected by electrophoresis using 1% ethidium bromide-stained agarose gel in 1×TAE buffer.

Real-time PCR using the Taqman® chemistry

A duplex real-time PCR assay for the simultaneous detection of a 102 base pairs (bp) and a 78 bp fragment of the S004 and S044 protein genes, respectively, and a single real-time assay for the detection of a 77 bp fragment of the S024 protein gene, were developed using the Taqman® chemistry. Primers and probes (Table 2) were designed from SGI-1 sequence of Typhimurium DT104 strain 96-5227 (GenBank accession number AF261825). BLAST searches were performed on the primer and the probe sequences to evaluate the assay specificity.

Amplifications were performed using an ABI PRISM 7500 Fast Sequence Detection System (Applied Biosystems, Warrington, UK).

The duplex assay was performed in 25 µl which included 5 µl of DNA, 12.5 µl of Taqman® Fast universal master mix (Applied Biosystems), 0.3 µM of each primers SGI04F, SGI04R, SGI44F, and SGI44R (Invitrogen), 0.04 µM of the SGI04P, and 0.06 µM of the SGI44P probes (Eurogentec, Seraing, Belgium).

The single assay was performed in 25 µl volumes which included 5 µl of DNA, 12.5 µl of Taqman® Fast universal master mix (Applied Biosystems), 0.3 µM of each primers SGI24F and SGI24R (Invitrogen), and 0.04 µM of the SGI24P probe (Eurogentec).

The cycle conditions for both assays were, 95°C for 20 sec followed by 40 cycles of 95°C for 3 sec, and 60°C for 30 sec.

SGI-1 fingerprinting

Ninety-five pairs of primers were designed from SGI-1 sequence of Typhimurium DT104, strain 96-5227, accession number AF261825 (Table 3). Each PCR was designed to amplify a 180–247 bp fragment. The amplicons (markers) were distributed across the entire SGI-1 sequence at intervals of approximately 500 bp (Fig. 1). The position, sequence, and predicted melting temperature of each primer are shown in Table 3. For each strain tested, a 96th primer pair, consisting of the primers S004F and S004R used in the real-time PCR, was included as a control for successful amplification. DNA extracts from the reference strain 96-5227 and from the control strain P5374140 were also subjected to the fingerprinting. Two sets of fingerprinting assays were performed with sterile distilled water in place of bacterial DNA as a negative control, to monitor contamination.

Each amplification was performed in 10 µl consisting of 5 µl of 10×diluted DNA extracts, 1×Gold PCR buffer (PerkinElmer, Foster City, CA), 0.2 mM of each dNTPs (GE Healthcare, Giles, UK), 1.5 mM $MgCl_2$, 1 µM of each primer (Operon, Koeln, Germany), and 0.2 U of AmpliTaq Gold DNA polymerase (PerkinElmer). Samples were subjected to an initial denaturation at 94°C for 9 min, followed by 33 cycles of 94°C for 20 sec, 54°C for 30 sec, and 72°C for 1 min in 384-well PCR plates on an MJ Tetrad thermocycler.

Table 1. Strains Used in This Study

Sending laboratory	Species/serotype	Source (phage-type)	Resistance to					Number of strain
			A	*C*	*S*	*Su*	*T*	
Bundesinstitut für Risikobewer-tungK, Berlin, Germany	*S.* Albany	Fd	A	C	S	Su	T	1
	S. Brandenburg	Fd	A	C	S	Su	T	1
	S. Heidelberg	Al	A	C	S	Su	T	2
	S. Java	Fd	A	C	S	Su	T	1
	S. Livingstone	Fd	A	C	S	Su	T	1
	S. Saintpaul	Fd	A	C	S	Su	T	3
	S. Typhimurium	Al (206 var)	A	C	S	Su	T	1
	S. Typhimurium	Al, fd (104 or var.)	A	C	S	Su	T	6
	S. Typhimurium	Al (104 or var.)	A	C	sens	Su	T	1
	S. Typhimurium	Fd (193)	A	C	S	Su	T	1
	S. Typhimurium	Al (RDNC)	A	C	S	Su	T	1
	S. Typhimurium	Al (NK)	A	C	S	Su	T	3
Total								22
Veterinary Medical Research Institute, Budapest, Hungary	*E. coli*/EPEC	Fd, Al	NK	NK	NK	NK	NK	18
	S. Infantis	Fd	NK	NK	NK	NK	NK	4
	S. Typhimurium	Al, fd (104 or var.)	A	C	int	Su	int	11
	S. Typhimurium	Al, fd (104 or var.)	A	C	int	Su	int	3
	S. Typhimurium	Al, Fd (RDNC)	A	C	int	Su	int	3
	S. Typhimurium	Fd (NK)	NK	NK	NK	NK	NK	1
Total								40
Danish Veterinary Institute, Copenhagen, Denmark	*E. coli*	Al	A	C	S	Su	T	4
	S. Worthington	Al	A	C	S	Su	T	1
Total								5
Statens Serum Institut, Copenhagen, Denmark	*S.* Newport	Hn	A	C	S	Su	T	4
	S. Stanley	Hn	A	C	int	Su	sens	1
	S. Typhi	Hn	A	C	S	Su	T	1
	S. Typhimurium	Hn (104 or var.)	A	C	S	Su	T	13
	S. Typhimurium	Hn (104 or var.)	A	C	S	Su	T	7
	S. Typhimurium	Hn (104 or var.)	A	C	int	Su	T	1
	S. Typhimurium	Hn (92)	A	C	S	Su	T	1
	S. Typhimurium	Hn (UNTYP)	A	C	S	Su	T	1
	S. Typhimurium	Hn (NK)	A	C	int	Su	T	1
Total								30
Health Protection Agency, Centre for Infection, London, United Kingdom	*Proteus mirabilis*	Hn	A	NK	NK	NK	NK	4
	Providencia	Hn	A	NK	NK	NK	NK	1
	Proteus vulgaris	Hn	A	NK	NK	NK	NK	4
	Shigella boydii	Hn	A	NK	S	Su	T	10
	Shigella dysenteriae	Hn	A	NK	S	Su	T	12
	Shigella flexneri	Hn	A	NK	S	Su	T	11
	Shigella sonnei	Hn	A	NK	S	Su	T	3
Total								45
Central Institute for Animal Disease Control, Lelystad, The Netherlands	*E. coli*	Fd, Al	A	C	NK	Su	T	35
	E. coli	Fd	A	int	NK	Su	T	1
	E. coli	Fd	A	int	NK	Su	sens	1
	E. coli	Fd	A	C	NK	Su	T	1
	E. coli	Fd	sens	C	NK	Su	T	1
	E. coli	Fd	sens	C	NK	Su	T	1
	S. Agona	Hn	A	C	NK	Su	T	1
	S. Albany	Hn	A	C	NK	Su	T	1
	S. Cubana	Fd	A	C	NK	Su	T	1
	S. Derby	Hn	A	C	NK	Su	T	2
	S. Derby	Hn	NK	sens	sens	sens	sens	1
	S. Derby	Hn	sens	C	NK	Su	T	1
	S. Derby	Hn	sens	sens	NK	Su	T	1
	S. enterica	Al	A	C	iS	Su	T	1
	S. Java	Hn	A	C	NK	Su	T	1
	S. Java	Hn	NK	C	NK	Su	T	1
	S. Mbandaka	Fd	sens	sens	NK	Su	T	1

(*continued*)

TABLE 1. (*CONTINUED*)

Sending laboratory	*Species/serotype*	*Source phage-type*	*Resistance to*					*Number of strain*
			A	*C*	*S*	*Su*	*T*	
	S. Mbandaka	Fd	A	C	NK	Su	T	1
	S. Mbandaka	Fd	sens	sens	NK	Su	iT	1
	S. Panama	Hn	A	C	NK	Su	T	1
	S. Rissen	Hn	A	C	NK	Su	T	1
	S. Saintpaul	Fd	A	C	NK	Su	T	1
	S. Typhimurium	Al, Fd, Hn (104 or var.)	A	C	NK	Su	T	6
	S. Typhimurium	Al, Fd, Hn (104 or var.)	A	C	int	Su	T	36
	S. Typhimurium	Fd (104 or var.)	A	sens	int	Su	sens	1
	S. Typhimurium	Al (104 or var.)	A	int	sens	Su	sens	1
	S. Typhimurium	Hn (206 var)	A	C	int	Su	T	1
	S. Typhimurium	Al (U309)	A	C	int	Su	T	2
	S. Typhimurium	Al (RDNC)	A	C	int	Su	T	1
	S. Typhimurium	Hn (UNTYP)	A	C	int	Su	T	1
Total								106
Departmento Patología Animal, Madrid, Spain	*E. coli*	Al or Fd	A	NK	sens	Su	T	8
	S. Derby	Al or Fd	A	NK	sens	Su	T	1
	S. Wien	Al or Fd	A	NK	sens	Su	T	1
Total								10
Unité de Caractérisation et d'Epidémiologie Bactérienne, Maison-Alfort, France	*S.* Albany	Fd	A	C	S	Su	T	2
	S. Bredeney	Fd	A	C	S	Su	T	1
	S. Indiana	Al	A	C	S	Su	T	1
	S. Java	Fd	A	C	S	Su	T	1
	S. Newport	Al	A	C	S	Su	T	1
	S. Saintpaul	Al, Fd	A	C	S	Su	T	2
	S. Typhimurium	Al, Fd (104 or var.)	A	C	S	Su	T	2
Total								10
Istituto Superiore di Sanità, Roma, Italy	*S.* Infantis	Hn	A	C	S	Su	T	6
	S. enterica	Hn	A	C	S	Su	T	4
	S. Typhimurium	Hn (104 or var.)	A	C	S	Su	T	29
	S. Typhimurium	Hn (104 or var.)	A	C	sens	Su	T	1
	S. Typhimurium	Hn (U309)	A	C	S	Su	T	1
	S. Typhimurium	Hn (UNTYP)	A	C	S	Su	T	2
	S. Typhimurium	Hn (RDNC)	A	C	sens	Su	T	1
	S. Typhimurium	Hn (RDNC)	A	C	S	Su	T	2
	S. Typhimurium	Hn (NK)	A	C	S	Su	T	1
	S. Typhimurium	Hn (NK)	A	C	S	Su	sens	2
Total								49
National Institute of Hygiene, Warsaw, Poland	*E. coli*	Hn	A	sens	S	Su	sens	1
	E. coli	Hn	A	C	S	Su	T	1
	S. Choleraesuis	Hn	A	sens	S	Su	sens	1
	S. Enteritidis	Hn	A	sens	sens	Su	sens	6
	S. Enteritidis	Hn	A	sens	S	Su	T	3
	S. Enteritidis	Hn	A	sens	S	Su	sens	2
	S. Hadar	Hn	A	sens	S	Su	sens	2
	S. Hadar	Hn	A	sens	S	sens	T	1
	S. Heidelberg	Hn	A	sens	S	Su	sens	1
	S. Infantis	Hn	A	sens	S	Su	T	1
	S. Java	Hn	A	C	S	Su	T	1
	S. Kentucky	Hn	A	sens	S	Su	T	1
	S. Oranienburg	Hn	A	sens	S	Su	T	1
	S. Saintpaul	Hn	A	sens	S	Su	T	2
	S. Thompson	Hn	A	sens	S	Su	T	2
	S. Typhimurium	Hn (104 or var.)	A	C	S	Su	T	1
	Shigella flexneri	Hn	sens	sens	S	Su	T	1
	Shigella flexneri	Hn	A	C	S	sens	T	2
	Shigella flexneri	Hn	A	C	S	Su	T	2
	Shigella sonnei	Hn	A	sens	S	Su	T	2
Total								34

(*continued*)

TABLE 1. (*CONTINUED*)

Sending laboratory	*Species/serotype*	*Source phage-type*	*Resistance to*					*Number of strain*
			A	*C*	*S*	*Su*	*T*	
Veterinary	*E. coli*	Al	A	C	S	Su	T	35
Laboratories Agency,	*E. coli*	Al	A	C	sens	Su	T	9
Weybridge,	*S.* Java	Al	A	C	S	Su	T	1
United Kingdom	*S.* Typhimurium	Al (151)	A	C	S	Su	T	1
	S. Typhimurium	Al (193)	A	C	S	Su	T	8
	S. Typhimurium	Al (193)	A	C	sens	Su	T	2
	S. Typhimurium	Al, Fd (104 or var.)	A	C	S	Su	T	11
	S. Typhimurium	Al, Fd (U288)	A	C	S	Su	T	24
	S. Typhimurium	Al (UNTYP)	A	C	S	Su	T	3
Total								94
Public Health Agency, Winnipeg, Canada	*S.* Typhimurium strain 96-5227	NK (DT104)	A	C	S	Su	T	1
Health Protection Agency, London, United Kingdom	*S.* Typhimurium strain P5374140	Hn (DT104)	sens	sens	sens	sens	sens	1

Al: animal; Fd: Food; Hn: Human origin; A: ampicillin; C: chloramphenicol; S: streptomycin; Su: sulphonamides; T: tetracycline. sens: sensitive; int: intermediate. Phage type 104 or var. included the following closely related phagetypes: DT104, 104b, 104C, 104L, 12, 120, 203, 208, 56var., U288, U302, U311. var: variant; RDNC: reaction does not conform; NK: not known; UNTYP: untypable; EPEC: enteropathogenic *E. coli.*

After thermocycling, each reaction was supplemented with 8 µl of SyBr loading dye (15%, w/v, Ficoll-400 (GE Healthcare), 0.1 mg/ml Bromophenol blue, 4×SYBR Green I (Molecular Probes Europe, Cambridge UK)), and analyzed by electrophoresis in 6% horizontal polyacrylamide gels (Genetix) in 0.5×TBE buffer.

PCR assays were set up using an automated liquid handling system to prepare 384-well plates (4 samples processed at a time) and load polyacrylamide gels for electrophoresis.

Pyrosequencing®

Sequencing of real-time PCR products was performed by Pyrosequencing (Biotage). This method was chosen since the amplicons to sequence were small in length (77–102 bp) and Pyrosequencing was therefore more suitable than traditional sequencing. Furthermore, Pyrosequencing gives direct sequence data for each DNA, which means that little interpretation is necessary compared to other methods. The method is also very accurate, rapid (less than 1 hr) and offers high throughput (96 samples in parallel).

Pyrosequencing was performed using a Pyro Gold SQA sample preparation kit and a PSQ 96MA analyser (Biotage, Uppala, Sweden) according to manufacturer's instructions. Twenty microliters of double-stranded biotinylated PCR product was made single-stranded using the Vacuum Preparation Tool (Pyrosequencing AB). Briefly, biotinylated products were bound to streptavidin coated sepharose beads and made single stranded with a denaturation solution.

TABLE 2. PRIMERS AND PROBES USED IN REAL-TIME PCRS FOR THE DETECTION OF SGI-1

Region detected	*Primer F/R; Probes P*	*Sequence 5′ → 3′ and fluorescent dyes*	*Position in AF261825*
S004 protein	SGI04F	GCCATGCCTCCAACTGATC	3,282–3,300
	SGI04R	CGCTTCAACGAATCCGCAC	3,365–3,383
	SGI04P	FAM-CTGTATCTTAGTGTTTTATCGCCAC-BHQ1	3,305–3,329
S024 protein	SGI24F	CTCCGCAAGCTTGGCTATC	20,358–20,376
	SGI24R	GCAACGAGGAATTGCTGGC	20,416–20,434
	SGI24P	FAM-CATCTGCAAGTCGTCCAAATGATC-TAMRA	20,381–20,404
S044 protein	SGI44F	GATCTGGTAGGCAGTGACG	42,308–42,326
	SGI44R	CTTCAGCTCGTCAGGTGTC	42,367–42,385
	SGI44P	YY-CCATCCTTACTATTACCTAGCGAC-BHQ1	42,331–42,354

Keys
FAM 6-Carboxyfluorescein
BHQ1 Blach Hole Quencher
TAMRA Carboxytetramethylrhodamine (quencher)
YY Yakima Yellow

Table 3. Primers Used to Detect 96 Markers in SGI-1 Fingerprinting

Marker	*Forward (F) primer*	*F primer position*	T_m	*Reverse (R) primer*	*R primer position*	T_m	*Product length*
1	GCGCAGCAAAGCTTAAGCG	74	60	CTAACCATAAGAGAACTTCC	263	56	190
2	CCAAAGACACAAGCAGAGC	517	58	GCTTCCAGACTCTTTACTGG	696	60	180
3	GCGGAATGTCGCTATGACG	1,021	60	CTCTCCCATAAGCTTGACG	1,236	58	216
4	GAATCTGGAACTTGCTGTCC	1,528	60	GCTTATCGTTTACTAAGCCG	1,738	58	211
5	GACAGCTACCAATCCTCGG	1,997	60	CTGACTACTGACGAGCTGC	2,182	60	186
6	GATGTAAACGATCCGAAAGC	2,522	58	CACTTACGATGGTAAGCCC	2,736	58	215
7	CTCTTGAACGAGGCAAACC	3,062	58	CTACTTGCTCAGTATCAGGC	3,242	60	181
8	CCGTAAACATGGCCTCTGG	3,528	60	GACAAGACGCCTCAGAACG	3,737	60	210
9	GTAAGATCCAACGTATGTGC	4,042	58	GAATCAATGACTGAACAAATCC	4,224	60	183
10	CAACAATCTGAGACACCACC	4,565	60	CACAAAGCACTGATTTCGC	4,751	56	187
11	CAGCTTTGGAATGCCAGGG	5,080	60	CTATGACCCATCGAAAGCC	5,287	58	208
12	GCGGAAACGATAGCATCCG	5,552	60	GCGTGTTGTAGATCAAAGC	5,770	56	219
13	CTGGAGATGCTGCATCAGG	6,075	60	CTAACATCGGAGCTGTTGG	6,284	58	210
14	CTATCATTGCTTCTCCATTGC	6,576	60	GCTCTATTCAAATCTTGGAGG	6,766	60	191
15	CTGCTGATAAGAATTGATCG	7,079	56	GCAGCGTACTGGCTATTGC	7,277	60	199
16	CTCTTCACTCAATTGAACGG	7,554	58	CTTGGTTATCTCGTCGTGG	7,748	58	195
17	CAAACTTCTTGGCCTATGG	8,077	56	CAATCCATAGAAGCTTCCGG	8,293	60	217
18	CCGCTTGGTCTTTACCTGC	8,644	60	CAGATAGCAATGGATACTGC	8,851	58	208
19	CTCCAAGACCATACTCTTGC	9,085	60	CCAGTGCTTTGTTTGCAGG	9,291	58	207
20	GCCTTTGCTATTGCACCGG	9,603	60	GGTAAGTCATTGTCAGATGG	9,793	58	191
21	CTACTATCCTGACTCTGCG	10,086	58	CTACGTTACTGATCGTGGG	10,289	58	204
22	CACGGTCCACTAAGTCTGG	10,585	60	CCTCATTTGAAGGCGTTGC	10,785	58	201
23	GAGTGAGCATGGTTTGTGC	11,065	58	CTGGCCTTTCGCAACAAGC	11,271	60	207
24	CCATTTCGTGATTTCTGAGC	11,551	58	CGTTACGGTAACTATTGAGG	11,765	58	215
25	CAGATCACTTAGCTTCCCG	12,080	58	CAAGCACAGACCATCATAGC	12,274	60	195
26	GGGATTAGTACTGTTGTTTGC	12,605	60	GCCTACCAAGAGATGATAGG	12,787	60	183
27	CAGTTGAACAATCTGATCTGC	13,097	60	GGTATTCATGAGACACAACG	13,276	58	180
28	CTGATCTGCATTCACAAACG	13,109	58	GATGGATAGACTATTCAACG	13,319	56	211
29	CAGCTACAGCCTTGTTCGC	13,695	60	GGAGCATCAAGCGATGTGG	13,911	60	217
30	GGATGCCTGCATCAATGGC	14,010	60	CAGTTTGAGCCATCACAGC	14,214	58	205
31	GAACTGGAACTGGCTTTGC	14,536	58	CATTCTTTAGCTCTATCCAGC	14,716	60	181
32	GCATCCACGGAACTGAACC	15,059	60	GATAGGAATCTACTGAATCGG	15,276	60	218
33	GTTTCAACAGCCAACTCACC	15,491	60	CATGCCTGAAGCCTAATCC	15,690	58	200
34	GTTTGGCGACAATCGTAGC	16,110	58	GCTGAGTATGTGCGATAGC	16,308	58	199
35	GGTGGATATGAGCTCTTCC	16,581	58	GGAATTGGGTCATGGTCGC	16,795	60	215
36	CTGGTGATCGGAACAGTGC	17,108	60	GAAGTGTTTCGGAAGCTTCC	17,321	60	214
37	CCATCGGTCATGAATAGTGG	17,578	60	CTGCGTTTCACTTACCACC	17,786	58	209
38	CTTGAGGCTCAAGGCTTCC	18,060	60	GACCAGGCTTCCATATCGC	18,265	60	206
39	GAACCTTACAACCTGAACG	18,594	56	GGCTTGTTGTTACATACGC	18,806	56	213
40	GCATGTGACTCATAGTCGG	19,055	58	GAATCAATTGATTCCCTTTGC	19,264	58	210
41	CTGGCATAACGGAAGCTGG	19,587	60	GTACAAGCATTCGTCTTACC	19,793	58	207
42	GGGAAGCCAATTCTTGTCC	20,079	58	GCTGTCTTGCACTGACTGC	20,267	60	189
43	GCCATGATGGCTCTTACCC	20,541	60	GATGACAAAGTACCTGTTCG	20,750	58	210
44	CGAGAGAAGTAGATCTCAGG	21,046	60	CTCAATGACGAGTAACGAGG	21,258	60	213
45	GGGATCTGTAAAGTAGCTGC	21,558	60	GATCCTACTGACCACATAGC	21,767	60	210
46	GTCTGAGCGCTGAATACGG	22,089	60	GATGAAACTGCACAGTATTCG	22,272	60	184
47	GTGATCTGAATGAATTGAACC	22,577	58	CTGGTAGGAGAGGTTATCG	22,785	58	209

48	CATTCGTGGAGTCCACTCC	23,099	60	CAGTAATGAAGATACTGTTGG	23,297	58	199
49	CCCAATAGTCTTGGTCAGG	23,548	58	CAAACTTGAAGTAGATAGGC	23,750	56	203
50	CTTCAGCCACTCTTGTTGC	24,098	58	CTTGCTGAAAGAGTTGATGG	24,298	58	201
51	CTCTATCTCCTCTCATTTGG	24,583	58	CGAGATAGAAACCAACATGG	24,796	58	214
52	GCTTCAGCAACCATCCTCC	25,055	60	GTTGTTTGTAGAGCAAGATGC	25,258	60	204
53	CTCACTTTGATTCTGTTGCG	25,675	58	GCTGAACGTGACGATCAGC	25,860	60	186
54	CTAGCAACATCAGATACAGG	26,057	58	CATGTGTTTGCTGCTTTGGC	26,241	60	185
55	GTTGAAACCCTAGCGTAGC	26,531	58	GTCGTTGCCTGATGGATCC	26,721	60	191
56	CGGATACTTCCGCTCAAGG	27,190	60	GGATCTGGATTTCGATCACG	27,385	60	196
57	GCAAACCCTCACTGATCCG	27,399	60	CCTTGCTGTTCTTCTACGG	27,599	58	201
58	GTAGAAGAACAGCAAGGCC	27,583	58	GCTTACCAACCGAACAGGC	27,764	60	182
59	CGTGCATTTGTACGGCTCC	28,087	60	CAAGGGTGACTTCTATAGC	28,268	56	182
60	GTAGTGCTTACGTTGTCCC	28,553	58	GATCTCGCCTTTCACAAAGC	28,768	60	216
61	CTGAAATCCATCCCTGTCG	29,103	58	GATTCAGAATGCCGAACACC	29,321	60	219
62	CAGCTTCCAACCGGAAACC	29,539	60	GAACCGCACAATCTCGTCG	29,749	60	211
63	CATGAGTTCACCCTTGTCC	30,140	58	CCCATCTGATTACTCCACG	30,350	58	211
64	GACATCCTCGCTTCACTGG	30,548	60	GAGACGCAATGACGAAAGC	30,761	58	214
65	CTCTGGATCAAGTCAAGACG	31,065	60	CGAAGGCAAAGCTGAATCC	31,244	58	180
66	GTATCGTCGGGACATTGGC	31,545	60	CGCCATTCACGAGGTTTCG	31,760	60	216
67	GTCCGGAACTCTCTCATCG	32,053	60	GCGTTGCTCTCTTATCGGG	32,265	60	213
68	GTCATTCACGTTCCTTCGC	32,525	58	CACAAGCTCACGCAGAAGC	32,734	60	210
69	CTCAACCATTGCCGATTCG	32,977	58	GTGATGAGTCTCCTTGAGG	33,184	58	208
70	GTGTTCCCGATTCTGTTGC	33,533	58	GACAATCCAAACCCAACCG	33,745	58	213
71	GTCTGTGAGTGGGATTTCG	34,554	58	GGAAGAAGCGCACTACAGG	34,766	60	213
72	GTCTGTGAGTGGGATTTCG	34,554	58	GAAGAAGCGCACTACAGGC	34,765	60	212
73	TCGCACGGCGCTTCTCC	34,915	58	GTGGATTTCATCGCCAAGC	35,119	58	205
74	CCACAGCATGTGGAAGTGG	35,642	60	GTGCTGAGCTTTCCGTACC	35,851	60	210
75	CTCTACCAACGCGTACAGC	36,122	60	GAACGCACCGATACGAACG	36,313	60	192
76	GTTCTGGTCTTCGTTGATGC	36,801	60	GCCTTCATCCGTTTCCACG	36,998	60	198
77	GCAATCACACTCGATGATGC	37,660	60	GTCGTATCCCTCAAATCACC	37,856	60	197
78	GATGGAACATTGCGGATCG	37,997	58	CCTTGTTAGCCTTATCAGCG	38,210	60	214
79	GTTGGCCTTCCTGTAAAGG	39,408	58	GGCATGATCTAACCCTCGG	39,590	60	183
80	GCATATCTGCACAAGCTCG	39,988	58	GTTCTGCGATGAAGGTTGG	40,201	58	214
81	CCTTTCGTGCGTGTGAAGG	40,479	60	CGAGTATTGAGCATAGTCG	40,662	56	184
82	CGCCTGGATGAAACCTACG	40,950	60	CGCTTTGTCGGTATTGAGC	41,144	58	195
83	CATGCTCAACCACCATTTCG	41,459	60	GGCGTAATGGCTGTTGTGC	41,663	60	205
84	GAGAGTTATCGAGCGATTGC	41,917	60	GAAGATTTGAACTGGGTTGG	42,128	58	212
85	CATGGGTCTAAACATAGTTCC	42,421	60	GAACGTTCACTGACGAGGG	42,626	60	206
86	GCTTTCTCACTTGCAGAAGC	43,023	60	GGGAATGATGTCCTTCTCG	43,241	58	219
87	GCAACAAGTCGTGATGTGG	43,440	58	CCATCTTCATTCTTTCGCC	43,650	56	211
88	GGGCTACAACTCGGATTGG	43,874	60	GTTACATTGTAAGACTTCCCG	44,065	60	192
89	GATGTACGTGCACCAATTCC	44,499	60	CTCAACTAATGATCCTGGGC	44,717	60	219
90	CAATTCAGTAAGGATAATTCTGG	44,964	62	CCAAATTTTACCCATTTGCGC	45,205	60	242
91	CATTGACGGCACCTTATCG	45,423	58	GTATGAAGATGAACTTGAGC	45,633	56	211
92	GATGTACTTTTGGGATATTGAGG	45,841	64	GTTATCGTTTGTTAGAGTTATACC	46,087	64	247
93	GTCAACCAGGGAACGAAGC	46,471	60	GTCACTTTCACTCTCTTACG	46,680	58	210
94	GATACAGGTGACCACACCC	46,516	60	GGTTGAAGCTGATCTGCGC	46,705	60	190
95	GTATCTGGTCGGTTTACCG	47,158	58	GTGAACAAAGCAGTGAGGC	47,354	58	197
96	GCCATGCCTCCAACTGATC	3,282	60	CGCTTCAACGAATCCGCAC	3,383	60	102

The pyrosequencing forward primers, 5′GATCTAACCTG TATC3′, 5′CTATCACGGCATCTG3′, and 5′GTGACGAC GGCCATC3′ for the sequencing of real-time assay amplicons obtained in the detection of the S004, S024, and S004 proteins, respectively, were added at a concentration of 0.35 μM each, annealed to the single-stranded template by heating to 80°C for 2 min and allowed to cool to room temperature. The plate was then placed into the Pyrosequencer™. The reagent cartridge was filled with each nucleotide, enzyme, and substrate mixture with the appropriate volumes calculated by the instrument.

Results

Detection of SGI-1 left and right junctions by gel-based PCRs

The primer pairs U7L12/LJR1 (left junction) and 104RJ/C9L2 (right junction with retronphage element) amplified products of approximately 500 bp. The primer pair 104RJ/104D, for the detection of the right junction lacking the retronphage element, amplified a fragment of approximately 450 bp.

Fragments of both SGI-1 junctions were amplified from DNA extracted from 53% of the following *Salmonella* serovars: 4/4 Albany, 2/6 Derby, 6/6 Java, 1/1 Kentucky, 4/5 Newport, and 128/195 Typhimurium and one isolate with the serotype O rough, i:1.2 (Table 4). A retronphage element, detected using primers 104RJ/C9L2, was found only in Typhimurium and in the above-mentioned *S. enterica*. DNA from one isolate of Typhimurium amplified only SGI-1 Left Junction fragment (Table 4).

The SGI-1 junctions were detected in both control strains (96-5227 and P5374140) but not in the strains of the following serovars: Agona, Brandenburg, Bredeney, Cholerasuis, Cubana, Indiana, Livingstone, Newport, Orianenburg, Panama, Rissen, Stanley, Typhi, Wien, and Worthington (one strain of each), Thompson (two), Hadar, Heidelberg, and Mbandaka (three strains of each), Derby and untyped *Salmonella* isolates (four strains of each), Saintpaul (eight), Enteritidis and Infantis (11 strains of each) and Typhimurium (66) (Table 4). SGI-1 junctions were not detected in any of the *E. coli*, *Shigella*, and *Proteus* isolates.

Detection of class 1 integron gene cassettes in isolates in which the SGI-1 junctions were detected

The amplicon set consisting of two gene cassettes of 1,000 and 1,200 bp was amplified from 90% of *Salmonella* isolates in which the SGI-1 junction fragments were present. These isolates were of serovars Derby, Java, and Typhimurium (Table 4), all showing the resistance pattern (R-type) ACSSuT (pentaresistance). The single amplicon of 1,200 bp was amplified from 4% of the DNA recovered from isolates of serovars Albany and Typhimurium (Table 4). Three of the Albany isolates showed the pentaresistance pattern, and one strain had the R-type ACSuT and was sensitive to streptomycin. Two Typhimurium isolates exhibited R-types ACSu and ASSu. The remaining 6% of *Salmonella* strains DNA amplified gene cassettes of various length: 1,600 bp from one Kentucky isolate of R-type ASSuT; 700 bp + 1,200 bp + 1,600 bp from four Newport isolates of R-type ACSSuT; 700 bp + 1,200 bp from two Typhimurium isolates, R-type ACSSuT and ACSuT; 1,000 bp + 1,200 bp + 1,600 bp from two Typhimurium isolates showing the pentaresistance phenotype (Table 4).

The two gene cassettes of 1,000 and 1,200 bp were detected in control strain 96-5227. No class 1 integron gene cassette was detected in the control strain P5374140.

Detection of class 1 integron gene cassettes in isolates in which the SGI-1 junctions were not detected

Class 1 integron gene cassettes with approximately 150, 700, 1,000, 1,200, 1,600, 1,800, and/or 2,500 bp were detected in 48.5% of *Salmonella* isolates negative for both SGI-1 junctions (Table 4). The most prevalent cassette amongst these isolates was of 1,600 bp (22% of these isolates). The 1,200 bp gene cassette fragment was detected only in one isolate of Agona, of R-type ACSSuT, together with a 1,000 bp gene cassette (Table 4).

Class 1 integron gene cassettes were detected in 34% of *E. coli*, 11% of *Proteus*, and 5% of *Shigella* isolates. The single gene cassette of 1,600 bp was detected in 44% of these isolates.

Application of real-time PCR and comparison with the left and right junctions detection by gel-based PCR results

All DNA extracts were subjected to real-time PCR detecting the genes for three SGI-1 proteins S004, S024, and S044.

The three genes were identified in all the strains in which both junctions of SGI-1 had also been detected (Table 4). The three genes were not detected in strains in which SGI-1 junctions had not previously been detected, except in five *Salmonella* strains described below. Gene fragments of the S004 and S024 proteins alone were amplified from DNA extracted from one Typhimurium isolate in which only the SGI-1 left junction was present (Table 4). The S004 protein gene only was detected in one Agona and three Derby isolates (Table 4). For convenience, these five strains will be designated as the "unconventional" strains. The "unconventional" Agona strain was the only one in which the SGI-1 junctions had not been detected and in which gene cassettes of 1,000 and 1,200 bp had been identified (Table 4). A class 1 integron gene cassette of 1,000 bp was detected in each of the three "unconventional" Derby strains (Table 4). As described above, the "unconventional" Typhimurium and Agona strains had the pentaresistance ACSSuT phenotype. The three "unconventional" Derby strains had different R-types SSuT, CSSu, and SSu.

In addition to the BLAST search performed on primers and probes, the specificity of the real-time assays was further tested. Real-time PCR amplicons, obtained from DNA extracted from two Derby and three Typhimurium isolates, in which the SGI-1 junctions and the three SGI-1 proteins were present, and from the five "unconventional" strains were sequenced using a pyrosequencing assay. Results showed that all the amplicon sequences matched 100% the relevant gene sequences of strain 96-5227 SGI-1, GenBank accession number AF261825.

SGI-1 fingerprinting

SGI-1 fingerprinting was developed to obtain information on the content of the island in the five "unconventional" strains, compared to SGI-1 in other strains. Strains subjected

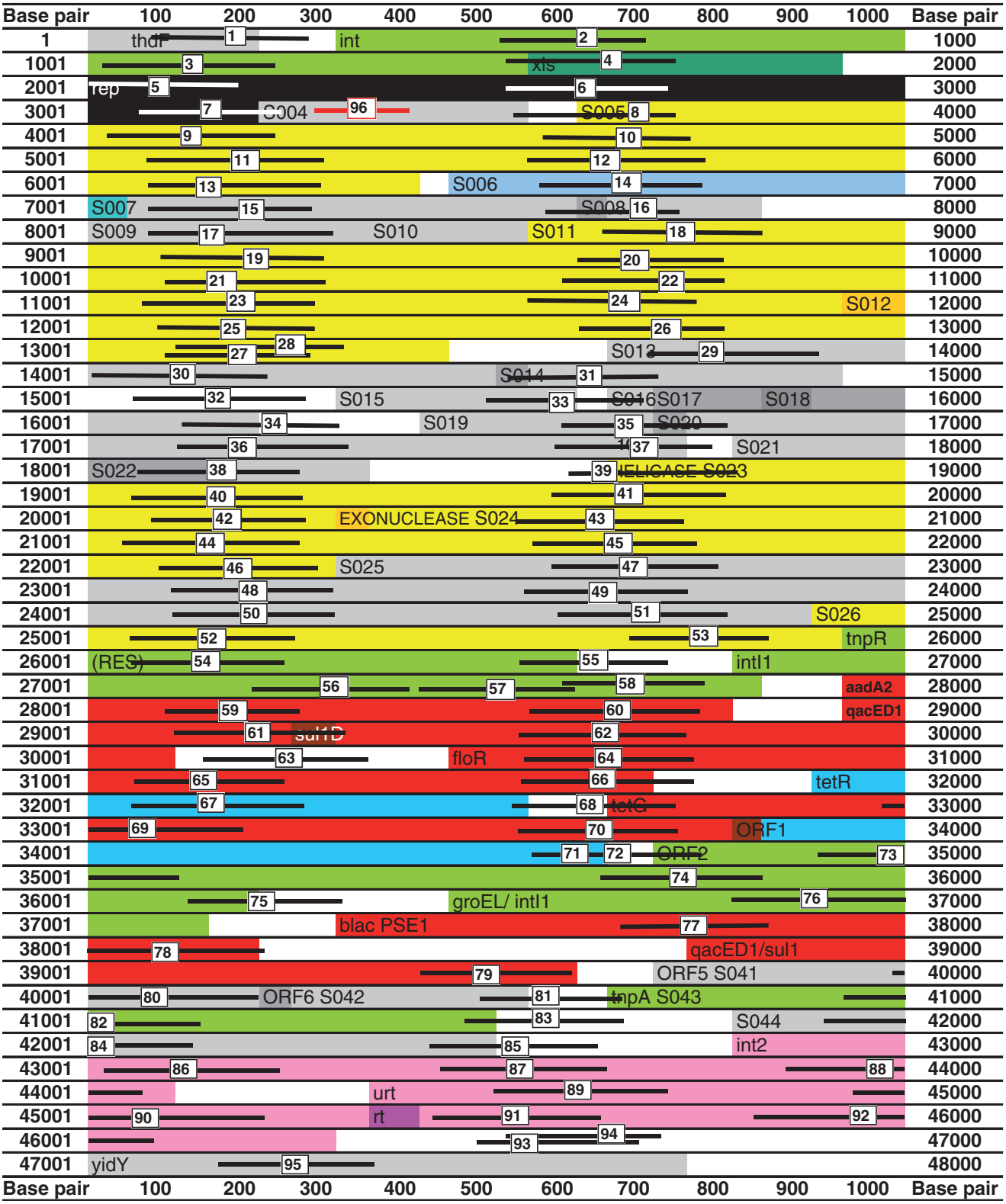

FIG. 1. Markers used in fingerprinting of SGI-1. The sequence is drawn in 1,000 bp segments, after the linear representation of SGI-1 by Boyd *et al.*,[5] and colored to indicate the locations of various features. The 96 amplicons (markers) used in fingerprinting are indicated by black bars. Marker 96 was used a positive control.

Table 4. Results of PCRs for the Detection of SGI-1 Left and Right Junctions, Class 1 Integron Gene Cassettes and from Real-Time PCRs for the Detection of Three SGI-1 Proteins

Strains and n *tested*	n *positive by gel-based PCR for SGI-1*				n *positive by real-time PCR for SGI-1 proteins*		
		Left junction	*Right junction*	*Class 1 integron gene cassette, amplicon lengths* (n)	*S004*	*S024*	*S044*
S. Agona	1	0	0	1,000 + 1,200 **(1)**	1	0	0
S. Albany	4	4	4	1,200 **(4)**	4	4	4
S. Brandenburg	1	0	0	None	0	0	0
S. Bredeney	1	0	0	1,600 (1)	0	0	0
S. Choleraesuis	1	0	0	1,600 (1)	0	0	0
S. Cubana	1	0	0	None **(1)**	0	0	0
		2	2	1,000 + 1,200 **(2)**	2	2	2
S. Derby	6	0	0	1,000 **(3)**	3	0	0
		0	0	1,600 (1)	0	0	0
				None (5)			
S. Enteritidis	11	0	0	1,000 (2)	0	0	0
				1,600 (4)			
S. Hadar	3	0	0	None (1)	0	0	0
				1,600 (2)			
S. Heidelberg	3	0	0	700 (2)	0	0	0
				1,600 (1)			
S. Indiana	1	0	0	1,600 (1)	0	0	0
				None (1)			
S. Infantis	11	0	0	1,000 (4)	0	0	0
				1,600 (1)			
				2,500 (5)			
S. Java	6	6	6	1,000 + 1,200 **(6)**	6	6	6
S. Kentucky	1	1	1	1,600 (1)	1	1	1
S. Livingstone	1	0	0	1,800 (1)	0	0	0
S. Mbandaka	3	0	0	1,000 (3)	0	0	0
S. Newport	5	4	4	700 + 1,200 + 1,600 **(4)**	4	4	4
		0	0	1,600 (1)	0	0	0
S. Oranienburg	1	0	0	1,600 (1)	0	0	0
S. Panama	1	0	0	None (1)	0	0	0
S. Rissen	1	0	0	None (1)	0	0	0
				700 (2)			
S. Saintpaul	8	0	0	1,600 (5)	0	0	0
				700 + 1,600 (1)			
S. Stanley	1	0	0	700 (1)	0	0	0
S. Thompson	2	0	0	None (1)	0	0	0
				1,600 (1)			
S. Typhi	1	0	0	None (1)	0	0	0
				1,200 **(2)**			
				700 + 1,200 **(2)**			
S. Typhimurium	195	128	128	1,000 + 1,200 **(122*)**	128	128	128
				1,000 + 1,200 + 1,600 **(2)**			
		1	0	1,000 + 1,200 **(1)**	1	1	0
		0	0	Variable (66) a	0	0	0
S. Wien	1	0	0	1,600 (1)	0	0	0
S. Worthington	1	0	0	None (1)	0	0	0
S. enterica	5	1[b]	1[b]	1,000 + 1,200 **(1)** b	1[b]	1[b]	1[b]
		0	0	None (3)	0	0	0
				150 (1)			
Total	445	147	146		151	147	146

[a]150 (2); 1,000 (2); 1,600 (8); 1,800 (3); none (51).
[b]O rough i:1.2.
N: number of strains. B: in bold are the strains subjected to the fingerprinting; *21 of these strains were subjected to the fingerprinting.

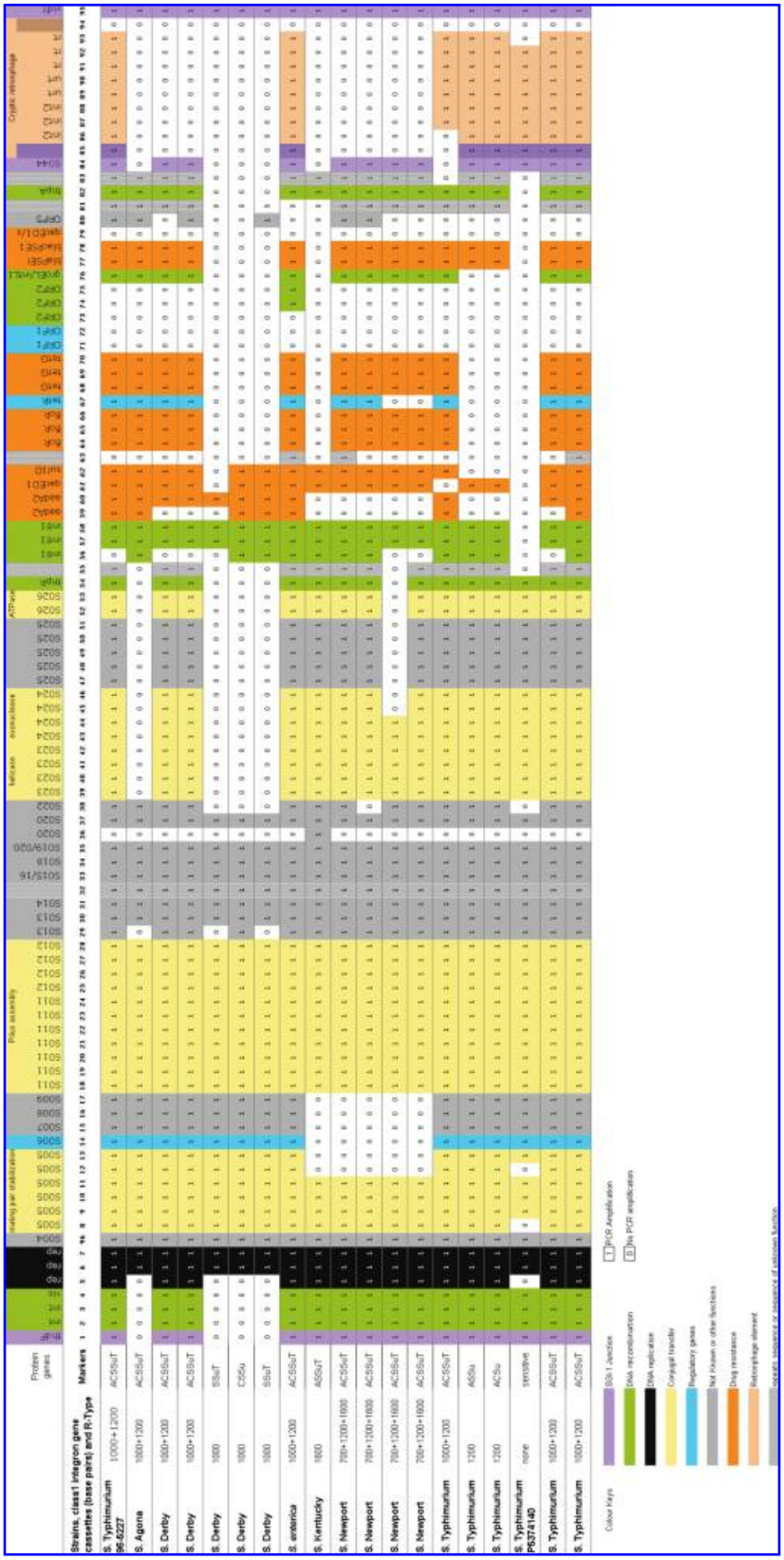

FIG. 2. Fingerprints of the *Salmonella* Genomic Island-1 in 19 *Salmonella* isolates. R-Type: antibiotic resistance; A: ampicillin; C: chloramphenicol; S: streptomycin; Su: sulphonamides; and T: tetracyclines. Drawn after Boyd *et al.* 2001 linear representation of SGI-1.[5]

to the fingerprinting are indicated in bold in Table 4. In addition, DNAs from 10 *E. coli*, nine *Proteus*, one *S. boydii*, two *S. dysenteriae*, five *S. flexneri*, two *S. sonnei* strains, and from control strains 96-5227 and P5374140 were subjected to the fingerprinting.

Figure 2 shows the alignment of SGI-1 fingerprints of 19 relevant strains. The aligned fingerprints were compared to the fingerprint obtained from the reference strain 96-5227.

Eighty-six (90%) of the PCRs were successful in showing a specific amplification using DNA recovered from the reference strain 96-5227 (Fig. 2). The unsuccessful markers and their location in SGI-1 can be seen in Fig. 2.

Because the MDR region is already known to be polymorphic and has been characterized previously,[4,10,14,19,29] this section will describe results obtained from outside the MDR region (non-MDR region).

One Agona and three Derby isolates ("unconventional" strains), one Kentucky and four Newport strains, and one Typhimurium strain ("unconventional" strain) all failed to amplify groups of consecutive markers detected in other isolates (Fig. 2); we therefore infer that the corresponding regions of SGI-1 are either absent in these isolates or have a highly divergent sequence. In the "unconventional" Agona and Derby isolates, the missing or divergent regions were situated between markers 1–5 and markers 39–55, respectively (Fig. 2). The region between markers 1–5 situated at SGI-1 left junction covered approximately 2,183 bp at the locus of genes coding for thiopene and furan oxidation (*thdF*, SGI-1 left junction), an integrase (S001), an excisionase (S002), and the 5′end of a replication protein gene (*rep*, S003) (Fig. 2). The second region, between marker 39–55, was approximately 8,662 bp and included genes encoding a helicase (S023), an exonuclease (S024), a "hypothetical protein" (S025), an ATPase (S026), and a resolvase (*tnpR* S027) (Fig. 2).

One Kentucky and four Newport isolates had one missing or divergent region situated between markers 12–17. These markers cover a region of approximately 2,742 bp and were situated at the 3′end of a gene coding for a mating pair stabilization protein (S005), genes for a regulator protein (S006), and three subsequent "hypothetical proteins" (S007, S008, and S009) (Fig. 2). In addition, one of the Newport isolate cited above had another missing or polymorphic region between markers 45–55. These markers spanned approximately 5,164 bp and included the 3′ end of a gene encoding an exonuclease (S024), genes for a "hypothetical protein" (S025), an ATPase (S026), and a resolvase (S027) (Fig. 2).

The fingerprint of the "unconventional" Typhimurium strain showed that the region between markers 83 and 86 were missing or divergent. These markers represent a fragment of approximately 1,783 bp at the SGI-1 right junction, which includes the locus of three consecutive repeat regions, the gene coding for a "hypothetical protein" (S044), two more repeat regions and part of the gene encoding the retronphage element prophage-like integrase (*int2*) (Fig. 2).

The fingerprint of the control Typhimurium DT104 strain P5374140 revealed that the strain had a similar SGI-1 to the reference strain 96-5227, except that the entire MDR region, from marker 55 to 83 representing a fragment of approximately 15,132 bp, was either missing or divergent (Fig. 2).

Markers from SGI-1 fingerprints were not detected in *E. coli*, *Shigella*, and *Proteus* strains, except for markers situated in the MDR region at the locus of the Class 1 integron integrase encoding gene (*Shigella*, *Proteus*, and *E. coli*) and *aadA2* and/or *floR* genes (*E. coli* only).

Discussion

SGI-1 is a mobilizable element that can be transferred between bacterial isolates and which carries a cluster of antibiotic resistant genes in its region.[5,12]

Previous PCR assays[4,10,14,19,29] for SGI-1 which rely on detecting the sequence of junctions between the island and the bacterial chromosome are not effective in non-*Salmonella* isolates,[1,12] and do not allow for possible divergence of SGI-1 from the single sequenced example.[5,6] In addition, in this study, primer pair 104RJ/104D for the right junction yielded amplimers of about 450 bp rather than 500 bp, as previously reported.[13,20] We therefore sought to develop novel rapid, specific real-time PCR assays which targeted sequences located well inside SGI-1 either side of the MDR region, and which were not dependent upon 100% sequence conservation at a single locus.

Real-time PCR assays

The real-time PCR assays were as effective as the earlier assays in detecting islands with conserved junction sequences. Moreover, they detected either one or two of the target genes in isolates where the junctions were not—or only partially—detected. Such "unconventional" isolates—undetectable by the earlier junction-dependant gel-based PCRs—may be clinically relevant: one (Agona) was found to contain SGI-1 typical class 1 integron 1,000 and 1,200 bp gene cassettes encoding for resistance to streptomycin and penicillins, respectively.

SGI-1 fingerprinting

The fingerprints showed that SGI-1 was polymorphic outside the MDR region, both between and within *Salmonella* serovars. These polymorphisms appear to be due to sequence divergence rather than deletions, since PCRs across the region did not yield the short products expected of a deletion (results not shown). The fingerprints also confirmed the results of the gel-based and real-time PCRs: isolates in which SGI-1 junctions or gene proteins were not detected, showed polymorphism in the corresponding region.

These polymorphisms were found at the SGI-1 junctions or/and within the backbone of the genomic island. The fingerprints of the "unconventional" Agona and Derby isolates showed polymorphism at the location of the right junction. This involves not only the *thdF* gene, but also the integrase and the excisionase gene and suggests that the *att*P site which represents SGI-1 specific sequence for recombination and the enzymes essential for integration into the chromosome[5,22] are either missing or different from that of the reference strain 96-5227. Similarly to the "unconventional" Derby isolates, a Typhimurium isolate lacking part of the left arm of the island has previously been described,[8] but in this strain the reminder of the island remained present. The fingerprint of the "unconventional" Typhimurium isolate showed that the markers located in the region of the S044 and the upstream region of the retronphage element (int2) were missing suggesting deletion or genomic variation at this section of the island. Deletions or polymorphisms found in the SGI-1 backbone, such

as in the Kentucky and Newport isolates in this study, or at the 3′end of the island, such as in the "unconventional" Typhimurium strain, have also been previously reported from multidrug resistant *Salmonella* isolates.[18,13] Insertion sequences and insertion sites usually surround the MDR region. Recombination events leading to gene cassettes replacements result in different antibiotic resistance profiles explaining the variability of the MDR region.[12] In the public health context, it might also be useful to understand genomic variations outside the MDR region, especially if these affect the lateral flow of SGI-1 in bacteria and whether additional functions, insertion sequences, transposon, or recombinase are present and responsible for variations in these strains.

One sensitive Typhimurium DT104 isolate showed the presence of a typical SGI-1, but lacked the entire MDR region. It is not possible to know whether this was due to deletion, or instead represents an ancestral SGI-1 which has not yet acquired an MDR region. To our knowledge such an event has not been reported before, perhaps because SGI-1 is not normally looked for in sensitive strains. Such "MDR-less" islands may be important to investigate if they predispose a sensitive strain to the acquisition of the MDR region and hence MDR phenotype.

Distribution of SGI-1 amongst multidrug resistant European Enterobacteriaceae isolates

SGI-1, or part of it, was detected in more than half of the *Salmonella* strains from nine countries and in seven serovars out of 27 tested. It was neither detected in the *Shigella*, *Proteus*, or *E. coli* strains nor in the multidrug resistant Saintpaul and Infantis, suggesting that SGI-1 is rare or absent in these organisms. There was not enough information about the origin and resistance phenotypes of the isolates to allow a thorough epidemiological study.

The detection of both class 1 integron 1,000 and 1,200 bp gene cassettes in an isolate was always linked to the presence of SGI-1, or part of it, and to the pentaresistance pattern. Neither the R-type ACSSuT nor the detection of the 1,000 bp gene cassette was always associated with the presence of SGI-1. Class 1 integron gene cassettes of various sizes were detected using PCR, but it was not possible to confirm whether these cassettes were within SGI-1. For this, appropriate assays should be performed amplifying products overlapping the integron(s) and SGI-1.

In summary, our three-target real-time assays appear to be a rapid and sensitive means of screening for SGI-1. The more complex fingerprinting assay also revealed the polymorphic nature of SGI-1, and argues strongly for the use of a multitarget assay for screening. Using these methods, an overview of the distribution of SGI-1 amongst multidrug resistant Gram-negative isolates from nine different European countries showed that the island was common in the *Salmonella* serovars Agona, Albany, Derby, Java, Kentucky, Newport, and Typhimurium, but rare or absent in other members of the Enterobacteriaceae family such as *Shigella*, *Proteus*, or *E. coli*.

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