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# Characterization of Variant *Salmonella* Genomic Island 1 Multidrug Resistance Regions from Serovars Typhimurium DT104 and Agona

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**Strains of multidrug-resistant *Salmonella enterica* serovar Typhimurium DT104 (DT104) and *S. enterica* serovar Agona (Agona) have been found to harbor *Salmonella* genomic island 1 (SGI1), a 43-kb genomic region that contains many of the drug resistance genes. Such strains are resistant to ampicillin (*pse-1*), chloramphenicol/florfenicol (*floR*), streptomycin/spectinomycin (*aadA2*), sulfonamides (*sul1*), and tetracycline [*tet*(G)] (commonly called the ACSSuT phenotype). All five resistance genes are found in a 13-kb multidrug resistance (MDR) region consisting of an unusual class I integron structure related to In4. We examined DT104 and Agona strains that exhibited other resistance phenotypes to determine if the resistance genes were associated with variant SGI1 MDR regions. All strains were found to harbor variant SGI1-like elements by using a combination of Southern hybridization, PCR mapping, and sequencing. Variant SGI1-like elements were found with MDR regions consisting of (i) an integron consisting of the SGI1 MDR region with the addition of a region containing a putative transposase gene (orf513) and *dfrA10* located between duplicated *qacEΔ1*/*sulI* genes (SGI1-A; ACSSuTTm); (ii) an integron with either an *aadA2* (SSu) or a *pse-1* (ASu) cassette (SGI1-C and SGI1-B, respectively); (iii) an integron consisting of the SGI1-C MDR region plus an orf513/*dfrA10* region as in SGI1-A (SGI1-D; ASSuTm; ampicillin resistance due to a TEM β-lactamase); and (iv) an integron related to that in SGI1 but which contains a 10-kb inversion between two copies of IS*6100*, one which is inserted in *floR* (SGI1-E; ASSuT). We hypothesize that the MDR of SGI1 is subject to recombinational events that lead to the various resistance phenotypes in the *Salmonella* strains in which it is found.**

In the 1990s, the prevalence of multidrug-resistant (MDR) *Salmonella enterica* serovar Typhimurium phage type DT104 (hereafter called DT104) has increased dramatically in the United Kingdom (45, 46), the United States (18, 22), and Canada (29). Many countries have also documented outbreaks associated with MDR DT104 in poultry, beef, cheese, and swine (11, 13, 19, 26, 48). Although case-control studies have suggested that MDR DT104 is more virulent than sensitive strains of DT104 or other *Salmonella* serotypes (15, 49), another report suggested that the percentage of bacteremia was no higher than with other *Salmonella* serotypes (42).

Nonetheless, encouraging findings have come from the United Kingdom which have documented a 22% decrease in 1999 from 1996 levels of DT104 displaying the multidrug resistance phenotype (43). MDR DT104 strains have been described as resistant to a core group of antimicrobial agents, including resistance to ampicillin, chloramphenicol/florfenicol, spectinomycin/streptomycin, sulfonamides, and tetracycline (commonly abbreviated ACSSuT). In addition to ACSSuT strains, others have been identified which are resistant to fluoroquinolones, trimethoprim, and aminoglycosides (12, 17, 29, 44). The majority of ACSSuT DT104 isolates have a similar MDR region in their genomes comprised of the *floR* and *tet*(G) genes bracketed by the *pse-1* and *aadA2* gene cassettes of two class 1 integrons clustered in a 13-kb region (2, 6, 28, 36, 38).

A number of *Salmonella enterica* serovar Agona (hereafter referred to as Agona) strains harboring the same antimicrobial resistance region have been characterized, suggesting horizontal gene transfer of this region (10). The element containing the MDR region, *Salmonella* genomic island 1 (SGI1), has been cloned from the genome of a Canadian isolate and comprises a 43-kb region between *thdF* and a novel retron sequence (5). Recently, the entire SGI1 element has been sequenced and, in addition to the MDR region, contains at least 25 or more open reading frames (ORFs), including an integrase gene and an excisionase gene, some of which showed similarity to genes commonly found on conjugative plasmids (4).

Among the members of the family *Enterobacteriaceae* and pseudomonads, transfer of antibiotic resistance genes is due largely to broad-host-range plasmids that carry transposons (14). Many of the resistance genes in the transposons have been found to be mobile gene cassettes carried as part of integrons (16). Four classes of integrons have been described based on the similarities between their integrases, but the majority described belong to class 1, and this type has been found in multiple *Enterobacteriaceae*, including *Escherichia coli*, *Citrobacter* spp., *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., and *Salmonella* spp. (14, 20, 27, 40, 50).

Class 1 integrons contain a 5′ conserved segment (5′-CS) which consists of the *intI1* gene, encoding the site-specific integrase, and the associated *attI1* site, the primary site of recombination, and the 3′ conserved segment (3′-CS) of variable length but generally consisting of *qacEΔ1*, encoding low-level resistance to some antiseptics; the *sul1* gene, encoding sulfonamide resistance; and *orf5*, a gene of unknown function (16). The gene cassettes, of which over 60 have been described

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TABLE 1. Strains used in this study and Southern hybridization data with various probes

| Strain | Serovar | Resistance phenotype[a] | Genomic island name | Size(s) (kb) of *XbaI* fragment(s) hybridizing[b] with: | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *aadA2* | *floR* | *tet*(G) | *pse-1* | *qac/sul1* | *tnpA*/S044 | p1-9 | |
| 96-5227 | DT104 | ACSSuT | SGI1 | 11.7 | 11.7 | 11.7 | 11.7, 4.3 | 11.7, 4.3 | 4.3 | 9.0, 4.1 | 29 |
| 959SA97 | Agona | ACSSuT | SGI1 | 11.7 | 11.7 | 11.7 | 11.7, 4.3 | 11.7, 4.3 | 4.3 | 9.0, 4.1 | 10 |
| 1169SA97 | Agona | ACSSuTTm | SGI1-A | 11.7 | 11.7 | 11.7 | 11.7, 8.8 | 11.7, 8.8 | 8.8 | 9.0, 4.1 | 4 |
| S/960725 | DT104 | ASu | SGI1-B | — | — | — | 2.5, 4.3 | 4.3 | 4.3 | 9.0, 4.1 | 4 |
| 0047SA97 | Agona | SSu | SGI1-C | 6.6 | — | — | — | 6.6 | 6.6 | 9.0, 4.1 | This study |
| S/954435 | DT104 | SSu | SGI1-C | 6.6 | — | — | — | 6.6 | 6.6 | 9.0, 4.1 | 4 |
| 953SA98 | Agona | ASSuTm[c] | SGI1-D | ND | ND | ND | ND | ND | ND | ND | This study |
| S/960081 | DT104 | ASSuT | SGI1-E | 9.1 | 9.1, 7.9 | 9.1 | 9.1, 7.9 | 9.1 | 9.1, 7.9 | 9.0, 4.1 | 4 |

[a] A, ampicillin; C, chloramphenicol (and flofenicol); S, spectinomycin and streptomycin; Su, sulfonamides; T, tetracycline; Tm, trimethoprim.
[b] —, no hybridization signal observed; ND, not done.
[c] Ampicillin resistance is due to the presence of a TEM β-lactamase.

(16, 35), consist of the coding region and the downstream 59-base element (59-be), which is responsible for recognition and mobilization of cassettes. The IntI1-catalyzed recombination between the *attI1* and 59-be sites is the main reaction responsible for inserting gene cassettes into the integron (33).

Transposon Tn*402* is a mobile class 1 integron that contains the 5′-CS and a transposition module consisting of four genes (*tniA*, *-B*, *-Q*, and *-R*) required for transposition (7). In addition, Tn*402* is bound by inverted repeats of 25 bp, IRi at the integrase end and IRt at the *tni* end. Several class 1 integrons appear to have originated from a Tn*402*-like ancestor by incorporation of the common part of the 3′-CS, including *qacE*Δ*1*, *sul1*, and *orf5*. Most of these integrons, though still bound by IRi and IRt, have lost part or all of the *tni* module and are deemed defective transposon derivatives (7).

Some of these integrons have been analyzed and shown to have an identical 5′-CS and 2-kb of the 3′-CS, after which this region diverges (21). One group, the In5 type, have lost parts of the 3′-CS and *tni* module, likely due to IS*1326*-mediated and/or IS*1353*-mediated deletions (7, 32). Another group, the In4 type, have a 3′-CS that includes a copy of IS*6100* but no transposition genes (34). In In4 itself, the IS*6100* element is flanked by short segments from the IRt end of Tn*402*, the outer one being 152 bp and the inner one 123 bp, in inverse orientation to one another. In other members of this group, only one or neither of the IRt ends may be present, and the IS*6100* element does not contain the partial copy (34). Thus, the SGI1 MDR region has a structure similar to that of an In4 integron (4, 5).

Since a number of DT104 strains have been identified which exhibit different resistance phenotypes, e.g., SSu, ASu, and ASSuT (3, 12, 36), we have examined a number of Typhimurium and Agona strains for the presence of variant SGI1s. Furthermore, the MDR regions were characterized to determine their genetic structure, and a nomenclature system is suggested for SGI1 variants.

## MATERIALS AND METHODS

**Bacteria and media.** The *Salmonella* strains used in this study are listed in Table 1. All strains were grown at 37°C in brain heart infusion broth or Luria-Bertani (LB) medium. Stock cultures were stored at −70°C in Microbank vials (Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada).

**Antimicrobial susceptibility testing.** Isolates were tested for susceptibility to various antimicrobial agents on Mueller-Hinton agar by the disk diffusion method as previously described (28). Disks containing: ampicillin at 10 μg, chloramphenicol at 30 μg, florfenicol at 30 μg, spectinomycin at 100 μg, streptomycin at 10 IU, sulfonamides at 200 μg, and tetracyclines at 30 IU were purchased from Sanofi Diagnostics Pasteur (Marnes-la-Coquette, France), except for disks with florfenicol, which were from Schering-Plough Santé Animale (Segré, France).

**DNA methodology.** Genomic DNA was isolated as previously described (5). Primers used in the study are listed in Table 2. Standard PCRs and long PCR were carried out as previously described (4). Southern blotting was carried out by standard methods (37) with probes labeled and detected by ECL kits using the manufacturer's instructions (Amersham Pharmacia Biotech). Probes were made by PCR using the primer pairs listed in Table 2.

**Computer-aided analysis and annotation.** Homology searches were carried out using the Blast suite of programs (1), and open reading frames (ORFs) were detected with ORFinder via the World Wide Web interface of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih).

**Nucleotide sequence accession number.** The complete nucleotide sequence of the region harboring the trimethoprim resistance determinant has been deposited in the GenBank database under accession number AY049746.

## RESULTS

DT104 and Agona strains isolated in Scotland and Belgium displaying the ACSSuT, ACSSuTTm, ASu, SSu, ASSuTm, and ASSuT phenotypes were characterized at the genetic level in this study. The strains were shown to possess the SGI1 or a variation thereof by using a combination of Southern blotting and PCR (Table 1). All strains studied displayed a PCR product using primers U7-L12 and LJ-R1 (left junction) and 104-RJ and C9-L2 or 104-RJ and 104-D primers (right junction), indicating the presence of the left and right SGI1 junction regions (Fig. 1). This result demonstrated that the SGI1 was located between *thdF* and a novel cryptic retronphage in all of the DT104 variants studied and located between *thdF* and *yidY* in the Agona strains, in which the retronphage is absent (5). In addition, all strains displayed an 8.9-kb and a 4.1-kb fragment when Southern blots of *Xba*I-digested genomic DNA were probed with p1-9, a probe spanning ORFs S023 to S024, suggesting the majority of the SGI1 is present (Fig. 1; Table 1) (4).

Thus, Agona 959SA97, previously shown to harbor the same MDR region as in ACSSuT DT104 (4), contains SGI1 (Fig. 1) (10). In the other strains studied, differences in SGI1 structure were revealed with Southern hybridization analysis of *Xba*I-digested genomic DNA using probes directed against genes found in the MDR region (Table 1). These variable MDR regions will be discussed in detail below.

TABLE 2. Primers used in this study

| Primer | Sequence (5′ to 3′) | Reference(s) | Description |
|---|---|---|---|
| U7-L12 | ACACCTTGAGCAGGGCAAG | 4, 5 | Left-junction PCR |
| LJ-R1 | AGTTCTAAAGGTTCGTAGTCG | 4, 5 | Left-junction PCR |
| 104-RJ | TGACGAGCTGAAGCGAATTG | 4, 5 | Right-junction PCR |
| C9-L2 | AGCAAGTGTGCGTAATTTGG | 4, 5 | Right-junction PCR |
| 104-D | ACCAGGGCAAAACTACACAG | 4, 5 | Right-junction PCR |
| aadA2-L | TGTTGGTTACTGTGGCCG | 29 | *aadA2* probe |
| aadA2-R2 | TGCTTAGCTTCAAGTAAGACG | This study | *aadA2* probe |
| StCM-L | CACGTTGAGCCTCTATATGG | This study | *floR* probe |
| St-CM-R | ATGCAGAAGTAGAACGCGAC | This study | *floR* probe |
| tetG-L | CAGCTTTCGGATTCTTACGG | 29 | *tet*(G) probe |
| tetG-R | GATTGGTGAGGCTCGTTAGC | 29 | *tet*(G) probe |
| pse-L | AATGGCAATCAGCGCTTCCC | 29 | *pse-1* probe |
| pse-R2 | ACAATCGCATCATTTCGCTC | This study | *pse-1* probe |
| QS-1 | ATGAAAGGCTGGCTTTTTCTTG | 4 | *qac/sul1* probe |
| QS-2 | TGAGTGCATAACCACCAGCC | 4 | *qac/sul1* probe |
| DB-T1 | TGCCACGCTCAATACCGAC | This study | IS*6100*/S044 probe |
| MDR-B | GAATCCGACAGCCAACGTTCC | This study | IS*6100*/S044 probe |
| MDR-7 | AACCGTGCATCTATCGAGC | This study | |
| F6 | TTGGAAACAGACGGCATGG | 10 | |

**MDR region coding for ACSSuTTm.** Agona 1169SA97 probed with *aadA2*, *floR*, or *tet*(G) showed the same sizes of hybridizing bands as for Agona 959SA97; however, when probed with *pse-1, qac/sul1*, or IS*6100*/S044, an 8.8-kb fragment hybridized with the probe, which was approximately 4.5 kb larger than the expected 4.3-kb *Xba*I fragment (Table 1). PCR of this region with primers pse-L and MDR-B produced a major band of 8.9 kb and a minor band of 4.4 kb (data not shown). Sequence analysis of the purified 8.9-kb product revealed a 2,868-bp region inserted between copies of the *qacE*Δ*1* and *sul1* genes that contains two other genes, *dfrA10*, encoding resistance to trimethoprim, and *orf513*, a putative transposase (Fig. 1). This structure is identical to one found in In7 (31).

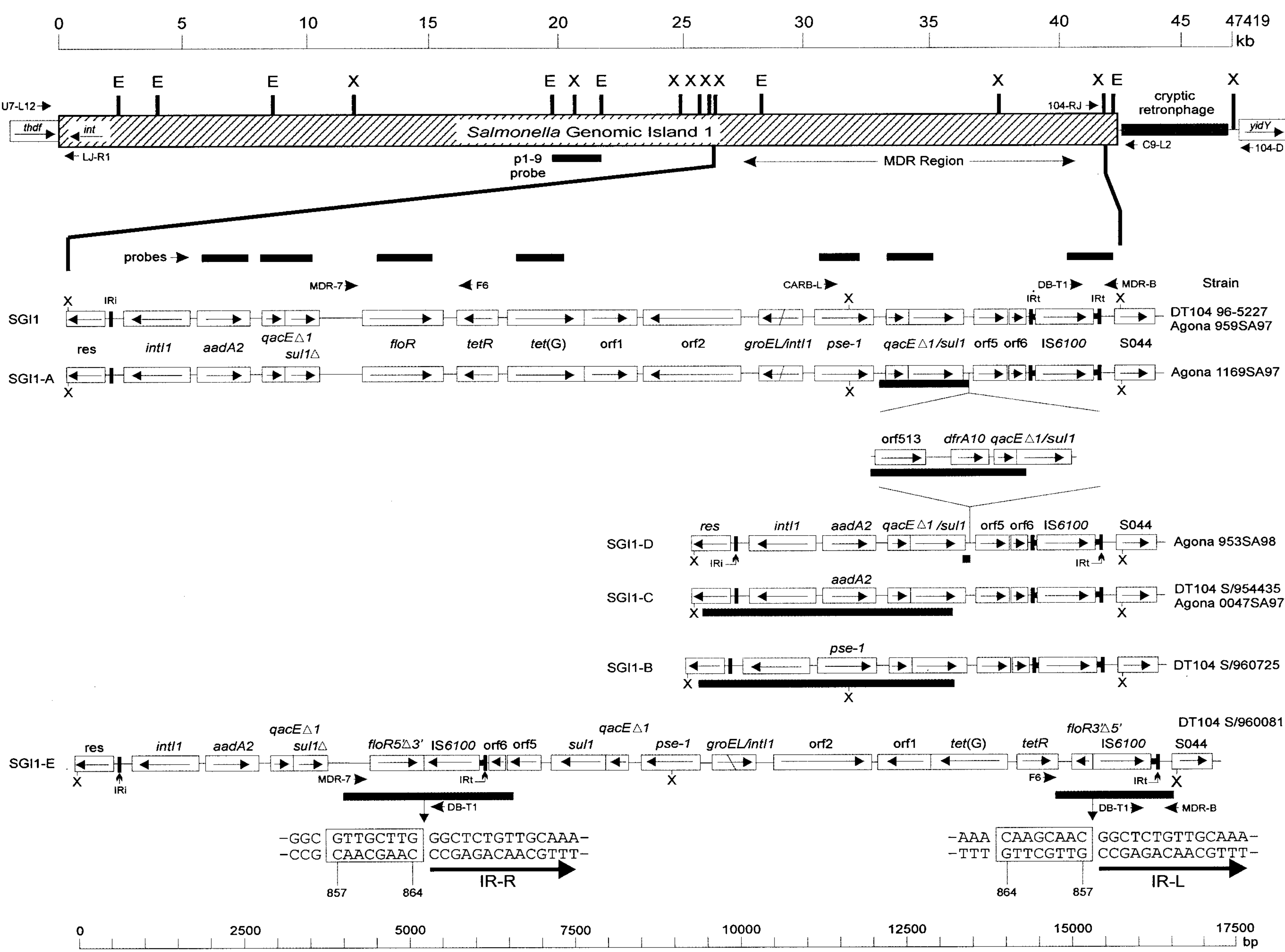
A 2,154-bp segment of this region, encompassing *orf513*, is also found upstream of the *cat* gene in In6 (31, 42), and the *ampC* and *ampR* genes in the integron in pSAL (47). The Orf513 protein is 65% identical to the OrfA protein, whose gene and a truncated version of it surround a *floR* gene from a plasmid found in *E. coli* isolated from cattle (9), and 55% identical to the Orf2 protein found in the SGI1 MDR region (4). An alignment of these proteins is shown in Fig. 2. All these proteins have been annotated as putative transposases or transposase-like, presumably due to homology to known transposases. Interestingly, all members of this group of proteins are found associated with resistance genes.

The size of a PCR product using the pse-L/MDR-B primer set is 4.4 kb with DNA from DT104 96-5227, the same size as a minor band amplified when using 1169SA97 DNA. Restriction enzyme analysis of these two amplicons revealed identical patterns (data not shown). Thus, it appears that a small subpopulation of Agona 1169SA97 cells may exist in which the *orf513/dfrA10* region is deleted. We propose to name the genomic island found in Agona 1169SA97 SGI1-A.

**MDR regions coding for ASu, SSu, and ASSuTm.** DT104 strain S/960725, which was resistant to ampicillin and sulfonamides (ASu), was shown to contain SGI1 sequences upstream of the MDR region by PCR and Southern hybridization (Table 1). Southern blots of *Xba*I-digested S/960725 chromosomal DNA probed with either *aadA2*, *floR*, or *tet*(G) did not produce a hybridization signal, suggesting that these genes were absent in this strain. The hybridization signals generated when *qac/sul1* or *pse-1* (contains a *Xba*I site) were used to probe the blot suggested a single integron was present in this MDR region (Table 1). Indeed, PCR and sequence analysis of this region in DT104 S/960725 revealed that a single complete *pse-1*-containing integron was present between the *res* and IS*6100* sequences (Fig. 1). We propose to name the genomic island found in DT104 S/960725 SGI1-B.

Two strains studied, Agona 0047SA97 and DT104 S/954435, displayed resistance to only two antimicrobials tested, streptomycin and sulfonamides (SSu). Southern blots of *Xba*I-digested genomic DNA did not reveal any sequences homologous to *floR*, *tet*(G), or *pse-1*, suggesting these sequences were absent in the strains (Table 1). Further probing of DT104 S/954435 and Agona 0047SA97 with *aadA2*, *qac/sul1*, or IS*6100*/S044 showed hybridization products of 6.6 kb, suggesting only one copy of *qac/sul1* was present and it was localized to the same *Xba*I fragment that contains the *aadA2* gene (Table 1). These results suggested a single integron may be present

FIG. 1. Genetic organization of the MDR regions of the various strains in this study based on Southern hybridization data, PCR mapping, and sequence analysis. A schematic of SGI1 is shown at the top, with the approximate locations of the primers used to detect the left and right junction regions indicated. The cryptic retronphage region is absent in Agona strains. Direction of transcription of genes is indicated by arrows. Locations of PCR products used as probes are indicated (thin black bars), as are the locations of some primers used in mapping. IRi and and IRt are 25-bp imperfect inverted repeats defining the ends of class 1 integrons. Regions sequenced are indicated under the various MDR regions by a thick black bar. The nucleotide sequence of the insertion point of the IS*6100* element in the *floR* gene of S/960081 is shown; the 8-bp region duplicated upon insertion is boxed (coordinates are for the *floR* gene only). IR-L and IR-R refer to the inverted repeats that define one end of IS*6100*. The sequence of the *orf513/dfrA10* region from Agona 1169SA97 has been assigned accession no. AY049746.


Salmonella Genomic Island 1
MDR Region
cryptic retronphage
p1-9 probe
probes
kb
47419
U7-L12
LJ-R1
104-RJ
C9-L2
104-D
thdf
int
yidY
Strain
SGI1
SGI1-A
SGI1-B
SGI1-C
SGI1-D
SGI1-E
res
intI1
aadA2
qacEΔ1
sul1Δ
floR
tetR
tet(G)
orf1
orf2
groEL/intI1
pse-1
qacEΔ1/sul1
orf5
orf6
IS6100
S044
orf513
dfrA10
floR5Δ3'
floR3Δ5'
sul1
MDR-7
F6
CARB-L
DB-T1
MDR-B
IRi
IRt
DT104 96-5227
Agona 959SA97
Agona 1169SA97
Agona 953SA98
DT104 S/954435
Agona 0047SA97
DT104 S/960725
DT104 S/960081
-GGC GTTGCTTG GGCTCTGTTGCAAA-
-CCG CAACGAAC CCGAGACAACGTTT-
857
864
IR-R
-AAA CAAGCAAC GGCTCTGTTGCAAA-
-TTT GTTCGTTG CCGAGACAACGTTT-
864
857
IR-L
bp

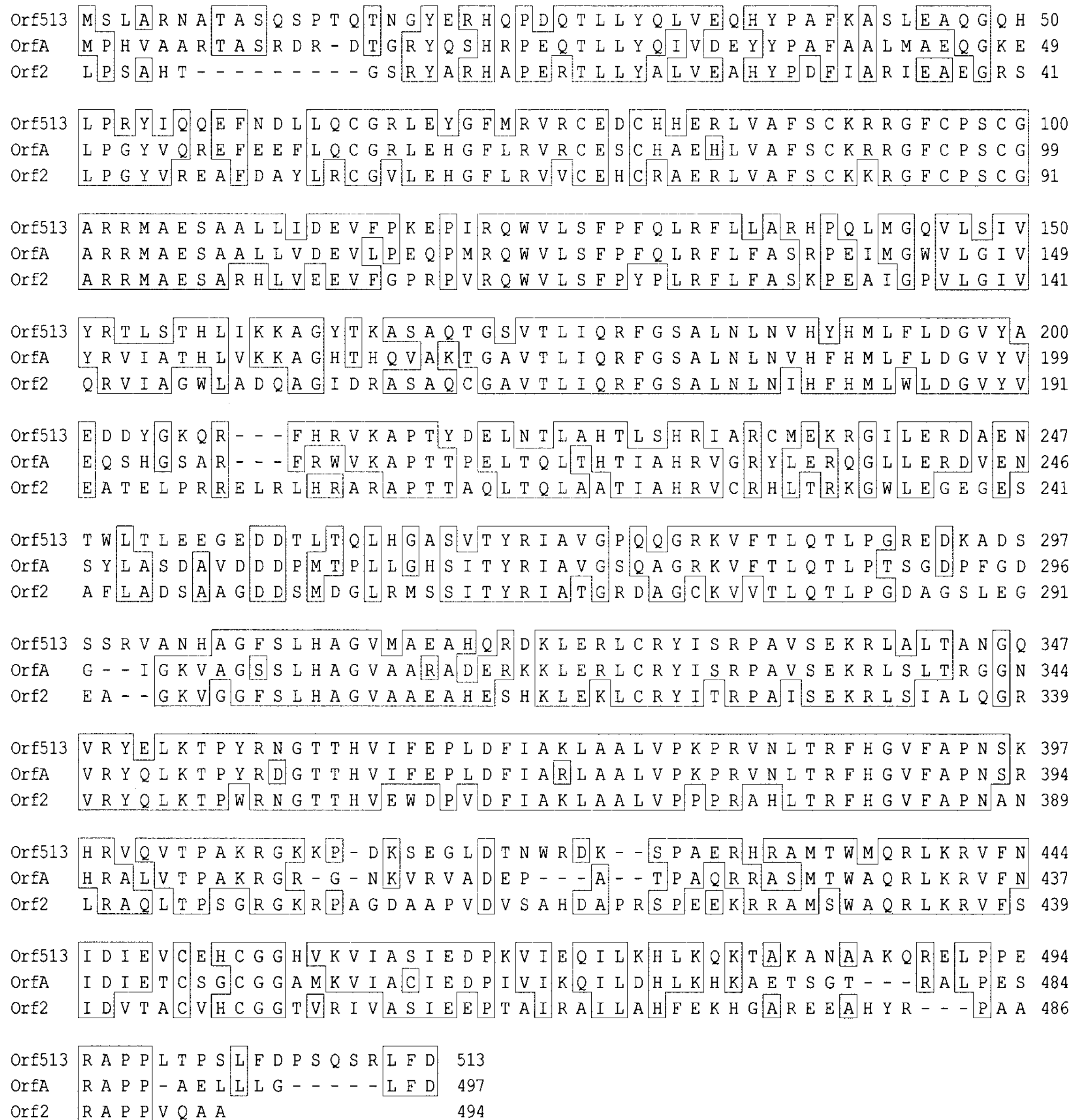


FIG. 2. Alignment of Orf513, OrfA, and Orf2 proteins. Identical residues in two or three of the proteins are boxed. Coordinates are at the right end of each line.

in the MDR region of these strains, and PCR and sequence analysis of this region in DT104 S/954435 confirmed that a single complete *aadA2*-containing integron was present between *res* and IS*6100* (Fig. 1). We propose to name the genomic island found in DT104 S/954435 and Agona 0047SA97 SGI1-C.

One strain, Agona 953SA98, was resistant to ampicillin, streptomycin, sulfonamides, and trimethoprim (ASSuTm). PCR analysis showed sequences from an *aadA2*-containing integron were present, but reactions with primers from *pse-1* were negative. However, PCR analysis with TEM-specific primers were positive, revealing that ampicillin resistance in this strain is due to a TEM-1-related β-lactamase (data not shown). Further PCR and sequence analysis showed that the trimethoprim resistance in Agona 953SA98 was due the presence of the same *orf513/dfrA10* region as found in Agona 1169SA97 (Fig. 1). We propose to name the genomic island found in Agona 953SA98 SGI1-D.

**MDR region coding for ASSuT.** Strain DT104 S/960081 displayed a resistance phenotype of ASSuT, and initial PCR analysis of the MDR region suggested all of the genes were present and in the same order as in ACSSuT strains, including the *floR* gene. However, PCR using the StCM-L/StCM-R primer pair did not produce a product, suggesting that either a mutated primer binding site was present in *floR* or a deletion existed within *floR* that encompassed a primer-binding site. Southern hybridization results, however, suggested that either IS*6100* or S044 was duplicated, as this probe hybridized with 7.9-kb and 9.1-kb *Xba*I fragments, as opposed to a single 4.3-kb fragment as in the ACSSuT strains (Table 1). The *floR* probe hybridized

to the same two fragments, while the *qac/sul1* probe hybridized only to the 9.1-kb fragment, suggesting a possible inversion had taken place within the MDR region which may have involved a portion of the *floR* gene and the IS*6100*-S044 region.

To test this hypothesis, PCRs using primer pair MDR-7/DB-T1 and primer pair F6/MDR-B were attempted, as both reactions were postulated to produce amplicons $<$2.0 kb in size if the inversion described above had occurred (Fig. 1). The MDR-7/DB-T1 reaction produced a 1.6-kb fragment, and the F6/MDR-B reaction produced a 1.9-kb fragment, both of which were sequenced. Analysis revealed that a second IS*6100* element had interrupted *floR*, and an inversion of the region between the two IS*6100* elements had taken place (Fig. 1 and Fig. 3). We propose to name the MDR region found in DT104 S/960081 SGI1-E.

## DISCUSSION

**Variant MDRs are In4-type integrons.** Original analysis of the DT104 SGI1 MDR region revealed an IS*6100* element in a position identical to that in In4 except for the absence of the partial IS*6100* copy (4, 5) (Fig. 1). Further analysis revealed the presence of IRi (positions 26637 to 26661 of the sequence with accession no. AF261825) between the *res* and *intI1* genes of the *aadA2* integron (Fig. 1). Thus, the entire MDR region of SGI1 (positions 26637 to 41698 of the sequence with accession no. AF261825) can be considered a single integron belonging to the In4 group (34). Additionally, this region is flanked by 5-bp direct repeats, ACTTG, strongly suggesting that it has been integrated by a transposition event. Hence, the MDR regions of the variant SGI1s described here can be described as In4-type integrons (34).

**Generation of variant MDRs.** SGI1 (ACSSuT) has been found in DT104, DT120, and Agona serovars of *S. enterica* (4, 5, 10). In addition, we have detected SGI1 in a strain of *S. enterica* serovar Paratyphi B isolated from a tropical fish (25). In every case, SGI1 is located at the end of the *thdF* gene, suggesting site-specific insertion possibly involving the product of the *int* gene found at one end of the genomic island (Fig. 1) (4, 5). Thus, it seems likely that whatever the donor strain, SGI1 exists on a transferable element and is acquired by other serovars via horizontal transfer. As the cryptic retronphage downstream of SGI1 has been found only in Typhimurium strains, it seems unlikely that transfer is mediated by phage transduction, though the resistance genes have been shown to be transduced (39).

However SGI1 is acquired, the following questions arise: what is the origin of the SGI1 MDR region and how are the variants generated? It seems reasonable to assume that the SGI1 MDR region evolved from insertions into an MDR region consisting of a single class 1 integron, i.e., SGI1-B (ASu) or SGI1-C (SSu). One scenario is that either SGI1-B or SGI1-C acquired another integron that included the *floR/tet*(G)*/orf1/orf2/groEL* region (*flo/tet*) as part of its 3′-CS, or acquired a second integron first, with subsequent integration of the *flo/tet* region between the two integrons. In either scenario, truncation of the *sul1* and *intI1* genes, as found in SGI1, would most likely have occurred during integration of the *flo/tet* region into the integron. The origin of the *flo/tet* region itself is unknown, and this combination of genes exists only in SGI1, though segments similar to parts of this region are found in various plasmids.

Homologs of *floR* ($>$95% identity) and the upstream sequence including the 99-bp direct repeat in SGI1 that brackets the *floR* gene (4, 6) have been found in plasmids from *Klebsiella pneumoniae*, *E. coli*, and *Photobacterium damselae* (8, 23). In the *P. damselae* and *E. coli* plasmids, the *floR* genes are associated with a putative transposase gene (*orfA*) whose product has homology to the product of *orf513* (Fig. 2) and to a lesser extent to the product of *orf2* of SGI1 (see above and Fig. 2). In the *K. pneumoniae* plasmid R55, the *floR* gene is followed by a gene whose product has 67% identity to the LysR-like Orf1 protein from the MDR region of SGI1 and a gene whose product has a C-terminal end identical to that of OrfA (8). Thus, all *floR* variants characterized to date are associated with putative transposase genes. As well, a 361-bp region highly similar to the region upstream of *floR* including the direct repeat sequence is found just upstream of a *tetR/tet*(G) region from *Pseudomonas* plasmid pPSTG1 (41). Thus, it can be speculated that the direct repeat sequence may play a role in the acquisition and/or mobility of genes in elements where it is found.

However the SGI1 MDR region originated, various mechanisms could account for the generation of the variant SGI-like elements. It may be that each type of MDR region has been assembled on another element (i.e., a resident plasmid) and then integrated into the genomic island in an insertional hotspot between the *res* gene and S044 in a strain already carrying the SGI1 progenitor in its genome. A similar explanation is that each type of SGI1-like element could have been assembled outside of the genome and then acquired by site-specific integration into the end of the *thdF* gene in a previously antibiotic-sensitive strain. However, the generation of the variant MDR regions from an SGI MDR region can most easily be postulated to have occurred by recombination between homologous regions (Fig. 3).

The structure of SGI1-A can be explained by the insertion of the *orf513/dfrA10* region via homologous recombination between the *qacE*Δ*1* and *sul1* regions, as has been suggested for In7 (42). This hypothesis is partially supported by the findings that deletion of the *orf513/dfrA10* region from SGI1-A can be detected by PCR, indicating this region may not be stable and lost without selective pressure. The simplest MDR regions are those containing single intact class 1 integrons, as was found in SGI1-B and SGI1-C (Fig. 1). It is important to note that these integrons are different from the two which exist in the SGI1 MDR region in that intact *intI1* and *sul1* genes are found in SGI1-B and SGI-C (Fig. 1) (4, 5). The generation of both single intact integrons in SGI1-B and SGI1-C from SGI can be explained by a single crossover between the copies of the *intI1* sequences or *qacE*Δ*1/sul1* sequences, respectively, with concomitant loss of the intervening DNA (Fig. 3). Similarly, SGI1-C and SGI1-D could be generated from SGI1-A by crossover between the different *qacE*Δ*1/sul1* copies (Fig. 3).

A single insertional event of the *orf513/dfrA10* element into SGI1-C would give rise to SGI1-D (Fig. 3). The origin of the *orf513/dfrA10* region is unknown, though the putative transposase Orf513 may be involved in its mobility and in the acquisition of different associated resistance genes (i.e., as in In6, In7, and pSAL). The SGI1-E variant likely arose from SGI1

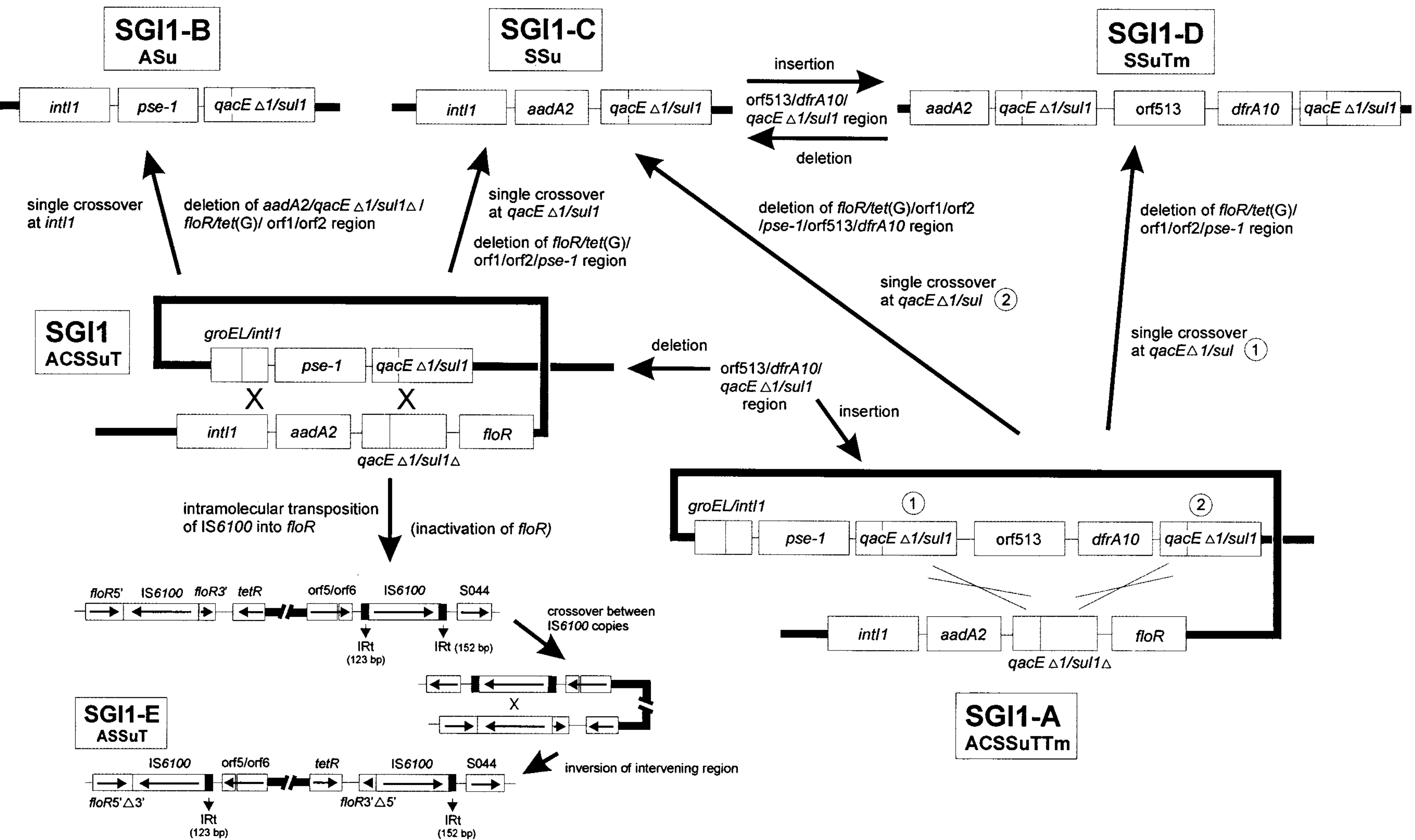



FIG. 3. Schematic diagram of the generation of variant SGI1 MDR regions from SGI1. Mechanisms are discussed in the text.

after intramolecular transposition of IS*6100* occurred (Fig. 3). The second copy inserted into *floR* in the opposite orientation to that of the other IS*6100* element, generating small direct repeats of the host target sequence (shown in Fig. 1), as has been observed with the insertion of many insertion sequence elements (24). Subsequently, a single crossover event between the two IS*6100* elements led to the inversion of the intervening region and the observed SGI1-E MDR region. Support for this hypothesis is provided for by the fact that each copy of IS*6100* in the SGI1-E MDR region is associated with only one of the IRt sequences that are found surrounding the single IS*6100* element in SGI1, the 123-bp region is found adjacent to the 5′ *floR* region, and the 152-bp region is found adjacent to the 3′ *floR* region (Fig. 1 and 3). A reverse scenario to produce the MDR region of SGI1, though possible, is unlikely to have occurred, as regeneration of an intact *floR* would necessitate deletion of one of the short direct repeats, and a copy of IS*6100* would also have to have been deleted.

**Conclusions.** This report describes the characterization of the MDR regions from a number of different DT104 and Agona strains which display antimicrobial resistance patterns other than the classical ACSSuT phenotype often described in strains involved in outbreaks. It will be interesting to see if strains containing other variant SGI1 elements coding for other resistance phenotypes can be isolated, e.g., ASSu, ASuTm, ACSu, CSSu, ACSuTm, and CSSuTm. Also, do MDR regions exist without at least one integron, i.e., CT, CTTm, C, T, CTm, or TTm? Knowing the relationship of the drug resistance phenotype to the type of SGI1 present may have important epidemiological implications if variants are isolated during the course of an outbreak situation involving an MDR *Salmonella* serovar containing an SGI1-like element. Investigators should also be aware that strains may exist with identical resistance phenotypes where a resistance may be due to different genes, such as ampicillin resistance due to the presence of a *pse-1*-containing MDR region in one strain but due to the presence of a TEM β-lactamase in the other strain.

In this report, we have initiated a nomenclature system to identify variants of the SGI1 regions in *Salmonella* strains. We encourage investigators to register any additional variants with us in order to maintain an ordered nomenclature system for SGI1 variants. Please contact the corresponding author with relevant information regarding novel SGI1 variants.

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