

## Variant *Salmonella* Genomic Island 1-L Antibiotic Resistance Gene Cluster in *Salmonella enterica* Serovar Newport<sup>∇</sup>

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**We report a new *Salmonella* genomic island 1 variant antibiotic resistance gene cluster called SGI1-L in a *Salmonella enterica* serovar Newport isolate containing a *dfrA15* gene cassette conferring resistance to trimethoprim. The isolate carried another class 1 integron containing the *aacC5* and *aadA7* gene cassettes conferring resistance to gentamicin and streptomycin/spectinomycin, respectively.**

A chromosomal genomic island called *Salmonella* genomic island 1 (SGI1) has been initially described in epidemic multidrug-resistant *Salmonella enterica* serovar Typhimurium phage type DT104 strains (2, 9). SGI1 contains an antibiotic resistance gene cluster and most often confers resistance to ampicillin, chloramphenicol/florfenicol, streptomycin/spectinomycin, sulfonamides, and tetracyclines.

The 43-kb SGI1 is located between the *thdF* and *int2* genes of the chromosome of *S. enterica* serovar Typhimurium. The *int2* gene is part of a retron sequence which has been reported to date only in *S. enterica* serovar Typhimurium (2, 9). In other *S. enterica* serovars, SGI1 is located between the *thdF* and *gidY* genes (2, 9). The antibiotic resistance gene cluster is located near the 3' end of SGI1 and constitutes a complex class 1 integron that belongs to the In4 group, which has been recently named In104 (6) (Fig. 1). At the first *attI1* site of this complex integron, the cassette carries the *aadA2* gene, which confers resistance to streptomycin and spectinomycin, and a 3' conserved segment (3'-CS) with a truncated *sull* (*sull* $\Delta$ ) gene is present. At the second *attI1* site, the cassette contains the  $\beta$ -lactamase gene *bla*<sub>PSE-1</sub>, conferring resistance to ampicillin, and the 3'-CS comprises a complete *sull* gene, conferring resistance to sulfonamides. Flanked by the two cassettes are the *floR* gene, which confers cross-resistance to chloramphenicol and florfenicol, and the tetracycline resistance genes *tetR* and *tet*(G). SGI1 has been recently demonstrated to be an integrative mobilizable element (4).

Variant SGI1 antibiotic resistance gene clusters have been described in a wide variety of *S. enterica* serovars (9). SGI1 variant antibiotic resistance gene clusters were accordingly classified in SGI1-A to SGI1-K (GenBank accession no. AY463797). These gene clusters were likely generated after chromosomal recombinational events or by antibiotic resistance gene cassette replacement at one of the *attI1* sites (9).

In this study, we examined an *S. enterica* serovar Newport strain, named 00-4093, isolated from stools from a French

patient with gastroenteritis. He had been infected by this strain while traveling to Egypt in 2000. Strain 00-4093 displayed the multidrug resistance profile typical of the SGI1 antibiotic resistance gene cluster but with additional resistance to gentamicin and trimethoprim.

Detection of SGI1, its location, and mapping of antibiotic resistance genes were performed by PCR using primers and conditions described previously (Fig. 1) (2, 4, 5). PCR results were positive for the left junction of SGI1 with the chromosomal *thdF* gene and for the right junction with the chromosomal *gidY* gene but negative for the junction with the *int2* gene. Thus, these results indicated that strain 00-4093 harbors SGI1 at the same chromosomal location, i.e., between the *thdF* and *gidY* genes, as in non-Typhimurium serovars. PCR mapping of the antibiotic resistance gene cluster showed the presence of fragments B, C, D, E, F, and *floR* of the sizes expected from the *S. enterica* serovar Typhimurium DT104 control strain BN9181 harboring SGI1, indicating the presence of the *floR*, *tetR*, *tet*(G), and *bla*<sub>PSE-1</sub> genes (Fig. 1). However, PCR A specific for the *aadA2* resistance gene cassette was negative. The positive result for PCR B nevertheless indicated the presence of the 3'-CS with the truncated *sull* gene. To assess the gene cassette array at the first *attI1* site of the SGI1 complex integron, a PCR with the forward primer of PCR A, targeting the *intI1* gene, and the reverse primer of PCR C, targeting the *tetR* gene, was performed (Fig. 1). Strain 00-4093 yielded a fragment of 4.4 kb that was cloned and sequenced (GenBank accession no. DQ647028). Sequence analysis identified the trimethoprim resistance gene cassette *dfrA15* at the first *attI1* site (Fig. 1). This *dfrA15* gene showed 99% nucleotide identity to those found in class 1 integrons from *Vibrio cholerae* and *Vibrio fluvialis* strains (GenBank accession no. AY605681, AB219235, AF221900, AB113114, and AY605692). The deduced amino acid sequence showed one amino acid change (I91T) compared with that of other DfrA15 proteins. This new variant SGI1 antibiotic resistance gene cluster was named SGI1-L according to the previously proposed nomenclature of SGI1 variants (9).

To identify the genes conferring resistance to gentamicin, streptomycin, and spectinomycin, PCR amplification of class 1 integrons using 5'-CS and 3'-CS primers was performed as described previously (10). This PCR yielded three fragments

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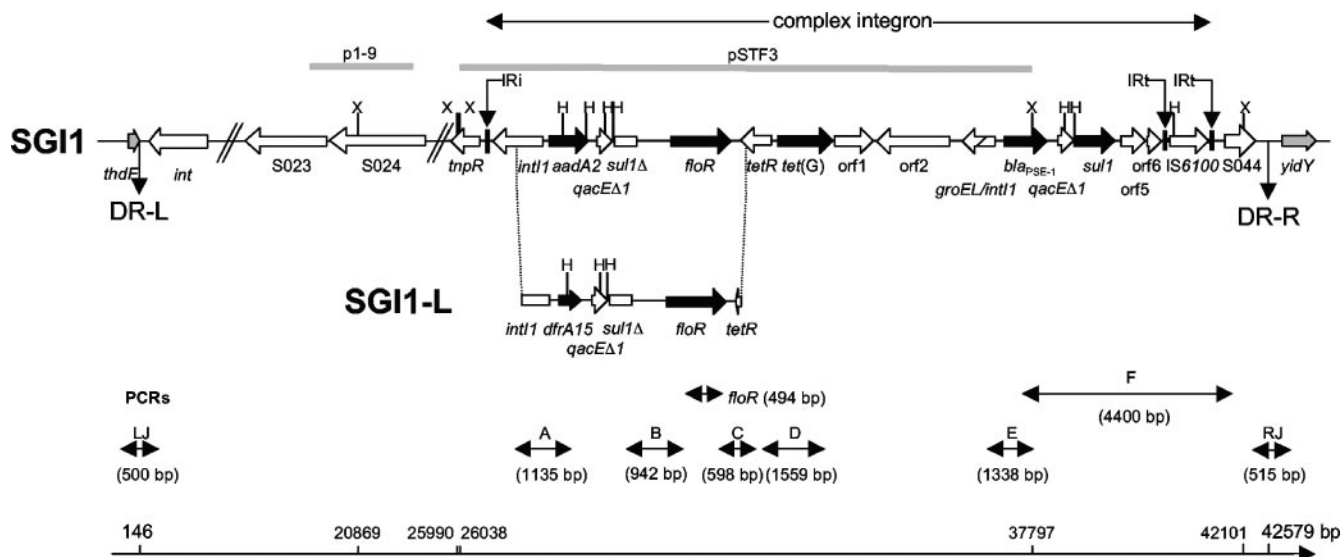


FIG. 1. Genetic organization of the antibiotic resistance gene clusters of SGI1 and the new SGI1-L variant of *S. enterica* serovar Newport strain 00-4093. The direction of transcription of genes is indicated by arrows. Black and gray arrows correspond to SGI1 antibiotic resistance genes and chromosomal genes flanking SGI1, respectively. DR-L and DR-R are the left and right direct repeats, respectively, bracketing SGI1. IRi and IRt are 25-bp imperfect inverted repeats defining the left and right ends of complex class 1 integrons. PCRs used to assess the genetic organization of the antibiotic resistance genes (PCRs *floR*, A, B, C, D, E, and F) and the SGI1 junctions to the chromosome (PCRs LJ and RJ [for left and right junctions, respectively]) are indicated. Abbreviations for restriction sites: X, XbaI; and H, HindIII. Gray thick bars indicate the p1-9 and pSTF3 probes. The positions of the DR-L, DR-R, and XbaI restriction sites are indicated on the scale according to GenBank accession no. AF261825.

with strain 00-4093, of which two were linked to the SGI1-L complex class 1 integron. The third fragment of 1.6 kb was cloned (plasmid pKP04) and sequenced (GenBank accession no. DQ647029). Sequence analysis identified the gene cassettes *aacC5*, also called *aac(3)-Id*, and *aadA7*, conferring resistance to gentamicin and streptomycin/spectinomycin, respectively (1, 3, 5, 7, 8). This gene cassette array was previously identified in SGI1-H of *S. enterica* serovar Newport and in SGI1-K of *S. enterica* serovar Kentucky (GenBank accession no. AY463797) (5). The class 1 integron containing *aacC5* and *aadA7* in strain 00-4093 was probably located elsewhere in the chromosome, since the gentamicin and streptomycin/spectinomycin resistances were not transferable and since PCRs using the 5'-CS and 3'-CS primers on plasmid extracts were negative (data not shown). This class 1 integron was also not located in SGI1-L, since PCR using a forward primer of S026 found upstream of the SGI1 complex integron and a reverse primer of *aacC5* was negative, whereas it was positive with SGI1-H carrying the *S. enterica* serovar Newport control strain (data not shown).

The presence of the 13-kb region found upstream of the SGI1 complex integron in strain 00-4093 was confirmed by Southern blot hybridization of XbaI-digested genomic DNA using the p1-9 probe (Fig. 1) (2, 4, 5). This probe corresponds to a 2-kb EcoRI fragment comprising parts of S023 and S024 open reading frames in the central region of SGI1 which encode putative helicase and exonuclease proteins (2). The p1-9 probe showed two XbaI fragments of the expected 4- and 9-kb sizes as in the SGI1-carrying control strains (Fig. 2). The new SGI1-L variant in strain 00-4093 was confirmed by Southern blotting of HindIII-digested genomic DNA with the pSTF3 probe containing nearly the entire SGI1 antibiotic re-

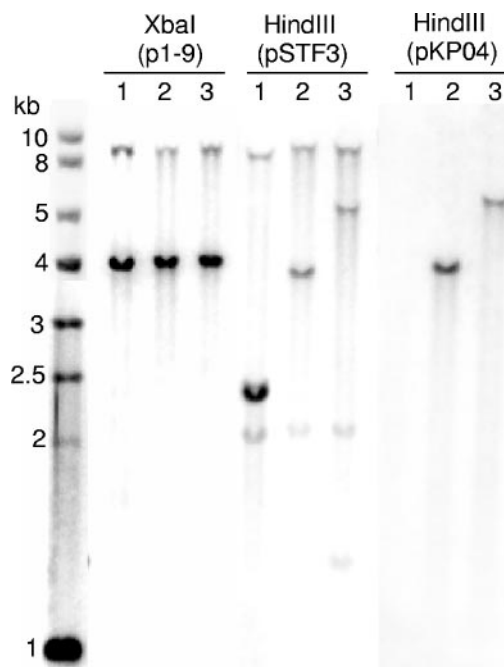


FIG. 2. Southern blot hybridization with the p1-9, pSTF3, or pKP04 probes of XbaI- or HindIII-digested genomic DNAs of *S. enterica* serovar Typhimurium DT104 strain BN9181 carrying SGI1 (lanes 1), *S. enterica* serovar Newport strain 01-2174 carrying SGI1-H (lanes 2), and *S. enterica* serovar Newport strain 00-4093 carrying SGI1-L (lanes 3). The 9- and 4-kb XbaI fragments obtained with the p1-9 probe correspond to XbaI site positions 11949 and 20869 and 20869 and 24937, respectively (as shown in Fig. 1 and according to GenBank accession no. AF261825).

sistance gene cluster of DT104 control strain BN9181 (Fig. 1 and 2). Probe pKP04 containing the *aacC5* and *aadA7* genes revealed a fragment of 6 kb in the HindIII-digested genomic DNA of strain 00-4093, suggesting as indicated above that these genes were not linked to SGI1-L (Fig. 2).

XbaI macrorestriction analysis by PFGE showed that strain 00-4093 was distinct from serovar Newport strains carrying SGI1-H which were also imported cases from Egypt (5; data not shown).

In conclusion, the SGI1 complex class 1 integron may contribute to the capture of a wide diversity of resistance gene cassettes in the *Salmonella* chromosome and thus may generate diverse antibiotic resistance gene clusters.

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