Whole-Genome Analysis of *Salmonella enterica* Serovar Typhimurium T000240 Reveals the Acquisition of a Genomic Island Involved in Multidrug Resistance via IS1 Derivatives on the Chromosome^{∇†}

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Salmonella enterica serovar Typhimurium is frequently associated with life-threatening systemic infections, and the recent global emergence of multidrug resistance in *S. enterica* isolates from agricultural and clinical settings has raised concerns. In this study, we determined the whole-genome sequence of fluoroquinolone-resistant *S. enterica* serovar Typhimurium T000240 strain (DT12) isolated from human gastroenteritis in 2000. Comparative genome analysis revealed that T000240 displays high sequence similarity to strain LT2, which was originally isolated in 1940, indicating that progeny of LT2 might be reemerging. T000240 possesses a unique 82-kb genomic island, designated as GI-DT12, which is composed of multidrug resistance determinants, including a Tn2670-like composite transposon (class 1 integron [*intI1*, *bla*_{oxa-30}, *aadA1*, *qacEΔ1*, and *sul1*], mercury resistance proteins, and chloramphenicol acetyltransferase), a Tn10-like tetracycline resistance protein (*tetA*), the aerobactin iron-acquisition siderophore system (*lutA* and *lucABC*), and an iron transporter (*sitABCD*). Since GI-DT12 is flanked by IS1 derivatives, IS1-mediated recombination likely played a role in the acquisition of this genomic island through horizontal gene transfer. The aminoglycoside-(3)-*N*-acetyltransferase (*aac(3)*) gene and a class 1 integron harboring the *dfrA1* gene cassette responsible for gentamicin and trimethoprim resistance, respectively, were identified on plasmid pSTMDT12_L and appeared to have been acquired through homologous recombination with IS26. This study represents the first characterization of the unique genomic island GI-DT12 that appears to be associated with possible IS1-mediated recombination in *S. enterica* serovar Typhimurium. It is expected that future whole-genome studies will aid in the characterization of the horizontal gene transfer events for the emerging *S. enterica* serovar Typhimurium strains.

Nontyphoidal salmonellae infections are frequent causes of bacterial food-borne gastroenteritis worldwide and are therefore an important public health issue. *Salmonella enterica* is a complex species composed of distinct groups of serovars that are defined by their antigenic structure and biotype. Presently, one of the most common serovars involved in human gastroenteritis is *S. enterica* serovar Typhimurium, which includes variants (pathovars) that are able to infect both livestock and human hosts (e.g., DT104), while others exhibit a restricted host range (e.g., DT2 and DT99) (31). Although *Salmonella* infections often present with self-limiting diarrhea (43), systemic diseases such as bacteremia and meningitis are frequently associated with highly invasive forms of nontyphoidal *Salmonella* such as serovar Typhimurium (16, 18).

Although life-threatening systemic infections caused by *S.*

enterica can in general be effectively treated by immediate antimicrobial therapy, the global emergence of multidrug resistance in *S. enterica* isolates from agricultural and clinical settings has raised concerns worldwide. Antibiotics are prevalent in agricultural settings since they are used not only to treat and prevent disease but also to promote growth in poultry and other livestock (28). As a result of widespread antibiotic use, multidrug resistance is commonly detected in *Enterobacteriaceae* isolated from animal sources, and the transmission of antimicrobial resistance determinants from veterinary sources to clinical settings via food contaminants has been documented (1, 4, 44). A recent report suggested that increasing ceftiofur resistance of *S. enterica* serovar Heidelberg isolates, which are often found in Canada, appears related to the levels of ceftiofur usage by poultry breeders (14), implying that ceftiofur use in chickens results in extended-spectrum cephalosporin resistance in bacteria isolated from both chickens and humans.

In the present study, we determined the whole-genome sequence of *S. enterica* serovar Typhimurium T000240 strain (previously designated as isolate “0” by Izumiya et al. [24]) isolated from a case of human gastroenteritis in 2000 in Japan (30). Strain T000240 is classified as DT12, which is an emerg-

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ing phage-type strain, and displays resistance to multiple antibiotics, including high-level fluoroquinolone resistance (24). A previous PCR detection study examined the antibiotic resistance and associated determinants of T000240 (24); in the present study, we further characterized a unique genomic island (GI), including a class 1 integron and composite transposons, by whole-genome analysis.

MATERIALS AND METHODS

Short-read DNA sequencing using an Illumina Genome Analyzer II. An ~500-bp DNA library of *S. enterica* serovar Typhimurium T000240 strain was prepared by using a genomic DNA Sample Prep Kit (Illumina, San Diego, CA), and DNA clusters were generated on a slide using a Cluster generation kit (v2) on an Illumina cluster station, according to the manufacturer's instructions. Briefly, to obtain $\sim 1.0 \times 10^7$ clusters for a single lane, the following series of reactions were performed using the standard Illumina protocols: template hybridization, isothermal amplification, linearization, blocking, denaturation, and hybridization of the sequencing primer. All sequencing runs for 80-mers were performed using an Illumina Genome Analyzer II (GA II) using an Illumina sequencing kit (v3). Fluorescent images were analyzed using the Illumina base-calling pipeline 1.4.0 to obtain FASTQ-formatted sequence data.

De novo assembly of short DNA reads. Prior to *de novo* assembly, obtained 80-mer reads were divided into 40-, 50-, 60-, or 70-mers from the 5' ends of 80-mer reads, followed by nucleotide trimming based on the phred quality value (cutoff of 14) using the Euler-SR "qualitytrimmer" command (9). These trimmed and read sequences were then assembled by using Euler-SR v1.0 (9) with the default parameters (vertex size, 25). N50 is a weighted median statistic such that 50% of the entire assembly was contained in contigs equal to or larger than this value.

Gap closing assisted by reference sequences. Reference-assisted gap closing was performed with OSLay v1.0 software (33) using the *S. enterica* serovar Typhimurium LT2 chromosomal DNA sequence as a reference genome (GenBank ID NC_003197.fna). First, homologous regions were identified between *de novo* assembly of short reads and LT2 chromosome DNA by BLASTN searches with $1E-10$ as a cutoff value (setting parameters: $-m\ 8 -e\ 1E-10$). Predicted supercontigs (an ordered and oriented set of contigs that contained gaps) were visualized by OSLay (33). Predicted gaps in supercontigs were amplified with a specific PCR primer pair, followed by Sanger DNA sequencing using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA).

Validation of gap closing and sequencing errors by short-read mapping. To validate whether misassembled sequences and incorrect gap closing remained after the reference-assisted gap closing, 40-mer short reads were aligned to the tentative complete chromosome DNA sequence of strain T000240 with Maq software (v0.7.1) using the "easyrun" Perl command (26). Read alignment for the validation of possible errors was performed by using the MapView graphical alignment viewer (5).

Annotation. NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP; <http://www.ncbi.nlm.nih.gov/genomes/static/pipeline.html>) was performed for the complete sequences of T000240 chromosome and plasmid DNAs. Several of the suggested errors were revised manually. Pseudogenes that were identified by GeneMarkS (6) were checked by the read-mapping correction described above. Genomic information, such as nucleic variations and circular representation, was analyzed by using IMC-GE software (Insilicobiology, Yokohama, Japan).

Extraction of SNVs from core genome sequences among *S. Typhimurium* strains. To identify single nucleotide variations (SNVs) of strain T000240 compared to the reference chromosome sequence of *S. Typhimurium* LT2 (NC_003197.fna), Maq software (v0.7.1) (26) was used with the easyrun command as the default parameter. Strain-specific SNVs were extracted from "cns.final.snp" files. To extract whole SNVs from the available genomic sequences of other *S. Typhimurium* strains, Maq software (v0.7.1) was used with the "Maq simulate" command with the following modifications of the default parameters: number of pairs of reads, " $-N\ 10000000$ "; mutation rate, " $-r\ 0$ "; and fraction of 1-bp indels, " $-R\ 0$ ". These parameters indicate that 20-million 35-mer hypothetical reads were generated with neither mutations nor indels from the genomic sequences used for SNV identification. SNVs located in prophages (Fels-1, Fels-2, Gifsy-1, and Gifsy-2) (27) were excluded from further phylogeny analysis.

Phylogenetic analysis. All SNVs were concatenated to generate a pseudo sequence for phylogenetic analysis. The DNA maximum-likelihood program

(RAxML v7.25) (38) was used for phylogenetic analysis with 1,000 times bootstrapping. FigTree v1.2.3 software was used to display the generated tree.

MLST analysis. The sequence type (ST) of T000240 was determined at the multilocus sequence typing (MLST) website for *S. enterica* (<http://mlst.ucc.ie/mlst/dbs/Senterica/>).

Pairwise alignment of GIs. Pairwise alignment of GI elements was performed by a BLASTN homology search (2) between the elements, followed by visualization of the aligned images with the ACT program (8).

Nucleotide sequence accession numbers. The short-read archive and the complete whole-genome sequence for strain T000240 have been deposited in the DNA Data Bank of Japan (DDBJ; accession numbers DRA000195 for the short reads, AP011957 for the chromosome DNA, AP011958 for plasmid pSTMDT12_L, and AP011959 for plasmid pSTMDT12_S).

RESULTS

Complete genome sequence of *S. Typhimurium* T000240. As a sequencing strategy, 13 million 80-mer short reads of the *S. Typhimurium* T000240 chromosome were obtained by Illumina GA II. To elucidate the length of short reads that would be effective for the *de novo* assembler program Euler-SR, the obtained reads were divided into 40-, 50-, 60-, 70-, and 80-mers from the 5' end. The best N50 results and minimum number of contigs were obtained using 50-mer short reads (see Fig. S1A in the supplemental material). This observation suggests that longer than 50-mer reads might include incorrectly assigned nucleotides with inaccurate phred quality value, leading to many more gaps by *de novo* assembly, whereas 40-mer reads seems to be too short for the ideal *de novo* assembly.

The contigs obtained using 50-mer reads were mapped onto the *S. Typhimurium* LT2 chromosome DNA sequence by OSLay software with BLASTN homology searches, and the tentative syntenic layout of individual contigs were organized as supercontigs (see Fig. S1B in the supplemental material). Potential gaps between contigs and supercontigs were filled by conventional PCR and Sanger DNA sequencing (data not shown). The complete sequences of the strain T000240 chromosome and plasmids were validated by remapping using the 50-mer short reads, and no SNVs or indels were found.

Genomic information of T000240. *S. enterica* serovar Typhimurium T000240 possesses 4,954,814 bp of chromosomal DNA (see Table S1 in the supplemental material and Fig. 1) and two plasmids (pSTMDT12-L: 106,510 bp; pSTMDT12-S: 8,670 bp) (see Table S1 and Fig. S2 in the supplemental material). MLST analysis revealed that T000240 is classified as ST19, which includes the *S. enterica* serovar Typhimurium strains LT2, DT104, SL1344, and 14028s. To further characterize the phylogeny of serovar Typhimurium strains, the 1,425 SNVs identified on the commonly shared genome sequence (see Table S2 in the supplemental material), excluding the prophages Fels-1, Fels-2, Gifsy-1, and Gifsy-2, were analyzed by the maximum-likelihood method. These analyses showed that T000240 was most similar to LT2, with 127 common SNV sites rather than the other multidrug-resistant strains DT104 and D23580 (untypeable phage type) (Fig. 2).

T000240-specific prophage genomic islands. To identify T000240-specific genomic islands, the complete chromosome DNA sequence of T000240 was pairwise-aligned with that of LT2 and D23580 (see Fig. S3 in the supplemental material). A total of five prophages were identified in T000240, including three (Fels-2, Gifsy-1, and Gifsy-2) of the four known prophages present in serovar Typhimurium strains; however, the

FIG. 1. Circular representation of the *S. enterica* serovar Typhimurium T000240 genome. From the outside inward, the outer circle 1 indicates the size in base pairs (Mb). Circles 2 and 3 show the positions of CDS transcribed in clockwise and anticlockwise directions, respectively (using color codes according to the COG classification table). Circle 4 shows genomic islands (yellow, GI-DT12; green, prophages). The purple bars on circle 5 indicate 13 *IS1* derivatives. The light blue bars on circle 6 and red bars on circle 7 indicate ribosomal DNA loci and tRNAs, respectively. Circle 8 shows a plot of G+C content (in a 0.5-kb window). Circle 9 shows a plot of GC skew ($[G - C]/[G + C]$; in a 0.5-kb window).

Fels-1 prophage harboring the *sodC* homolog was not found. Compared to LT2, the other two identified prophages were T000240 specific. The first was a seroconverting prophage (41,390 bp; nucleotides [nt] 368827 to 410216; attachment sequence ATTCGTAATGCGAAGGTCGTAGGTTTCGACT CCTATTATCGGCACCAGTTAAATCAA) carrying the *gtrABC* genes encoding seroconverting proteins, which was nearly identical to ST104 phage (AB102868) of *S. enterica* serovar Typhimurium DT104 (40) with only five mismatched nucleotides. The second prophage (28,165 bp; nt 2803437 to 2831601; attachment sequence CCTTCGGGCGCGTTTT) lacked notable features.

Characterization of *IS1* on T000240-specific genomic island GI-DT12. Pairwise alignments between the chromosomal DNA sequences revealed that an 82-kb genomic island (GI-DT12; nt 4007849 to 4090055) appeared to be acquired through the insertion of a large composite transposon on the strain T000240 chromosome (see Fig. S4 in the supplemental material). Southern blot analysis for class 1 integron with *BlnI* digestion revealed the presence of 150- and 80-kb positive bands, corresponding to the predicted bands from the chromosome and plasmid pSTMDT12_L, respectively (see Table S3 in the supplemental material). Interestingly, the insertion of GI-DT12 causes an insertional mutation of an eight nucleotide direct repeat (DR) (CATTTTCC) in a putative regulatory protein gene, leading to the formation of two truncated open

FIG. 2. Phylogenetic tree based on core genome SNVs among whole-genome sequenced *S. enterica* serovar Typhimurium strains using the maximum-likelihood method with 1,000-fold bootstrapping. The scale indicates that a branch length of 0.04 is four times as long as one that would show a 1% difference between the nucleotide sequences at the beginning and end of the branch. The number at each branch node represents the bootstrapping value. The sequence type (ST) 19 and 313 groups, as determined by MLST, are indicated by red and green circles, respectively. The SNV information is summarized in Table S2 in the supplemental material.

reading frames (ORFs; STMDT12-C38420 and STMDT12-C39440). Both the junctions and the elements of GI-DT12 were verified by PCR walking (data not shown).

Thirteen *IS1*-like elements were identified in the T000240 chromosome; six of these were located on GI-DT12. The nucleotide alignments and phylogenetic analyses revealed several notable features of these *IS1*-like elements: (i) four were *IS1* derivatives (*IS1*-1 to *IS1*-4), (ii) five of the six elements on GI-DT12 were *IS1*-2 to *IS1*-4 with 93% identities to the other *IS1*-1 elements, and (iii) the remaining eight *IS1*s, including one located on GI-DT12, were identical *IS1*-1 elements (Fig. 3 and see Fig. S5 in the supplemental material). GI-DT12 was flanked by two *IS1* elements (*IS1*-1g and *IS1*-2c), which shared 93.3% nucleotide identity in a 768-bp region. Such sequence

FIG. 3. Phylogenetic analysis of the 13 *IS1* derivatives identified, revealing four types of *IS1* sequences. Alignment of the nucleotide sequences are shown in Fig. S5 in the supplemental material.

divergence among GI-DT12-related *IS1* elements (*IS1*-2, -3, and -4) suggests that GI-DT12 was likely acquired in distinct events from that of *IS1*-1.

Generally, *IS1* transposition generates a DR in the flanking sequence. Among the eight *IS1*-1 elements identified in the T000240 chromosome, identical DR sequences were found in three elements (*IS1*-1b, *IS1*-1c and *IS1*-1d) located at both ends of the transposition but were not found in the other five elements. A number of ORFs flanking a few of the *IS1*-1 elements were likely to have been deleted by the transposition event (see Fig. S6 in the supplemental material), including the LT2 ORFs STM0339 to STM0343 near *IS1*-1a involved in fimbria synthesis, the phage-related ORFs near *IS1*-1e, the LT2 ORFs STM3600 (putative sugar kinase) and STM3601 (putative phosphosugar isomerase) near *IS1*-1f, and the LT2 ORFs corresponding to STM4295-STM4300 involved in the arginine cycle, tricarboxylic acid cycle, and melibiose uptake near *IS1*-1h.

Multidrug resistance encoded by T000240-specific genomic island GI-DT12. The sequence analysis of the T000240-specific genomic island GI-DT12 revealed the presence of several multidrug resistance determinants, including a composite Tn2670-like (class 1 integron [*intI1*, *bla*_{oxa-30}, *aadA1*, *qacEΔ1*, and *sul1*], mercury resistance proteins [*mer* operon], and chloramphenicol acetyltransferase [*cat*]) and Tn10-like (tetracycline resistance protein *tetA*) transposons (Table 1 and Fig. 4A). The region of these multidrug determinants, which was located between *IS26* to *IS1*-2, was identical to the *S. enterica* serovar Typhimurium plasmid pUO-StVR2, which is a hybrid virulence resistance plasmid that is widely distributed among isolates of *S. enterica* serovar Typhimurium (Fig. 4A) (20, 21). In addition to the class 1 integron present in pUO-StVR2, a *bla*_{oxa-30} gene cassette was inserted upstream of *aadA1*.

Our analysis also revealed that nearly all of the GI-DT12 region, with the exception of the class 1 integron and an iron transporter operon (*sitABCD*), was identical to plasmid pRSB107 isolated from an uncultured bacterium in a sewage-treatment plant sample (39) (Fig. 4A). In addition, an aerobactin iron-acquisition siderophore system (*lutA* and *lutABC*) (12) and an iron transporter operon (*sitABCD*) (34) were flanked by two *IS1*-2 elements, and a possible transposon was also identified in plasmid pCVM29188_146 of *S. enterica* serovar Kentucky from poultry (15) and plasmids in avian pathogenic *Escherichia coli* (11, 25).

Multiple antibiotic resistance encoded on plasmids pSTMDT12_L and pSTMDT12_S. T000240 harbors two plasmids: the small plasmid pSTMDT12_S and the large plasmid pSTMDT12_L. The large plasmid had a sequence nearly identical to that of plasmid pSLT of *S. enterica* serovar Typhimurium LT2 and was also found to have an antibiotic resistance composite transposon flanked by three *IS26* elements inserted into the putative resolvase corresponding to ORF PSLT045 in pSLT. The composite transposon included a possible aminoglycoside-(3)-*N*-acetyltransferase AAC(3) gene and a class 1 integron (Table 2 and Fig. 4B). The same *aac*(3) gene has been identified in *S. enterica* serovar Typhimurium G8430 (10), *Citrobacter freundii* (17), and *Klebsiella pneumoniae* (NC_013951.1). The class 1 integron of pSTMDT12_L differed from the integron located in GI-DT12, since a *dfrA1*

gene cassette encoding trimethoprim resistance was integrated into the plasmid integron.

The sequence analysis of small plasmid pSTMDT12_S revealed it was nearly identical to the sequence of plasmid pRSC15 of *S. enterica* serovar Typhimurium DT9 isolates (42). Both pSTMDT12_S and pRSC15 carried the sulfonamide resistance gene *sul2* and the streptomycin resistance genes *strA* and *strB* (Fig. 4C). These two plasmids appear to have originated from plasmid RSF1010 (36), which has been detected in many bacterial species since its first isolation in the early 1970s (19).

DISCUSSION

We first identified *S. enterica* serovar Typhimurium T000240 from a human source in 2000, which represented the first isolation of a strain displaying high-level fluoroquinolone resistance in Japan (30). Whole-genome analysis performed in the present study identified several T000240-specific genetic features, such as genomic islands, ISSs, and composite transposon elements, related to its highly resistant phenotype.

For decades, phage typing has been applied as an epidemiologic tool for the characterization of *S. enterica* serovar Typhimurium strains (3). However, recent innovations in DNA sequencing technologies have enabled the relatively simple acquisition of whole-genome information, which can then be rapidly characterized to determine differences of core genome sequences based on comparative analysis. Besides MLST and phage typing (3), whole-genome analysis may represent a practical approach to predicting strain lineages for further classification of Typhimurium strains. T000240 displays high sequence similarity to LT2 strain, even though LT2 was originally isolated in 1940 (27), suggesting that multidrug-resistant progeny of LT2 might be reemerging alongside DT104 and other definitive phage-type strains.

Comparative genome analysis showed that T000240 possesses the unique genomic island GI-DT12 on the chromosome (Fig. 4A). Notably, GI-DT12 is flanked by typical *IS1* elements, which are predominantly found in *Escherichia coli* (35) and *Shigella* spp. (23). Although *IS1* derivatives are present in plasmid pU302L of *S. enterica* serovar Typhimurium G8430 (10) and plasmid pAKU_1 of *S. enterica* serovar Paratyphi A AKU_12601 (22), our study represents the first finding of *IS1* derivatives integrated in the chromosome of *S. enterica* serovar Typhimurium.

In all, 13 *IS1* derivatives were identified in the T000240 chromosome. Since these repeat units generate assembly gaps during *de novo* assembly, gaps must be individually closed by a specific PCR; however, such efforts revealed that several of the 13 *IS1* derivatives had unique sequences. Interestingly, eight *IS1* (designated *IS1*-1) had identical sequences and were scattered on the chromosome, whereas the other five *IS1* (designated *IS1*-2 to -4) located on GI-DT12 showed somewhat less similarity to *IS1*-1 (Fig. 3), implying that these two groups of *IS1* derivatives have been independently acquired by horizontal gene transfer. This finding is intriguing because multiple, distinct *IS1* derivatives were identified on the chromosome, and the integration generated numerous gene disruptions and deletions adjacent to the insertion site (see Fig. S6 in the supplemental material). Although many of the target

TABLE 1. ORFs in genomic island GI-DT12

Gene_ID	Location (nt positions)	Product	COG category ^a
STMDT12_C38430	4007904–4008179	Insertion element IS1 protein InsA	1.4 [L] Replication, recombination, and repair
STMDT12_C38440	4008098–4008601	IS1 transposase	1.4 [L] Replication, recombination, and repair
STMDT12_C38450	Complement (4008595–4009548)	Hypothetical protein	5 Unannotated
STMDT12_C38460	4009929–4010159	VagC	4.2 [S] Function unknown
STMDT12_C38470	4010156–4010572	VagD	4.1 [R] General function prediction only
STMDT12_C38480	4010647–4012212	Putative ATP-dependent endonuclease of the OLD family	1.4 [L] Replication, recombination, and repair
STMDT12_C38490	4012197–4013219	Hypothetical protein	1.4 [L] Replication, recombination and repair
STMDT12_C38500	Complement (4013275–4013439)	Hypothetical protein	5 Unannotated
STMDT12_C38510	Complement (4013473–4013976)	Insertion element IS1 protein insB	1.4 [L] Replication, recombination, and repair
STMDT12_C38520	Complement (4013895–4014170)	Insertion element IS1 protein	1.4 [L] Replication, recombination, and repair
STMDT12_C38530	Complement (4014550–4016748)	Aerobactin siderophore ferric receptor protein IutA	3.7 [P] Inorganic ion transport and metabolism
STMDT12_C38540	Complement (4016833–4018110)	l-Lysine 6-monooxygenase	3.8 [Q] Secondary metabolites biosynthesis, transport and catabolism
STMDT12_C38550	Complement (4018107–4019849)	IucC	3.8 [Q] Secondary metabolites biosynthesis, transport and catabolism
STMDT12_C38560	Complement (4019849–4020796)	Aerobactin siderophore biosynthesis protein IucB	1.1 [J] Translation, ribosomal structure, and biogenesis
STMDT12_C38570	Complement (4020797–4022521)	Aerobactin siderophore biosynthesis protein IucA	3.8 [Q] Secondary metabolites biosynthesis, transport and catabolism
STMDT12_C38580	4022657–4023850	Transposon function, ShiF, putative	3.2 [G] Carbohydrate transport and metabolism
STMDT12_C38590	Complement (4024230–4024610)	Camphor resistance protein CrcB	2.1 [D] Cell cycle control, cell division, chromosome partitioning
STMDT12_C38600	Complement (4024951–4025385)	Enolase	3.2 [G] Carbohydrate transport and metabolism
STMDT12_C38610	Complement (4025853–4026710)	SitD	3.7 [P] Inorganic ion transport and metabolism
STMDT12_C38620	Complement (4026707–4027564)	SitC	3.7 [P] Inorganic ion transport and metabolism
STMDT12_C38630	Complement (4027561–4028388)	SitB	3.7 [P] Inorganic ion transport and metabolism
STMDT12_C38640	Complement (4028388–4029302)	SitA	3.7 [P] Inorganic ion transport and metabolism
STMDT12_C38650	4029658–4029933	Insertion element IS1 protein	1.4 [L] Replication, recombination, and repair
STMDT12_C38660	4029852–4030355	Insertion element IS1 protein insB	1.4 [L] Replication, recombination, and repair
STMDT12_C38670	Complement (4030535–4030786)	Hypothetical protein	5 Unannotated
STMDT12_C38680	Complement (4031182–4031538)	Hypothetical protein	1.4 [L] Replication, recombination, and repair
STMDT12_C38690	4032284–4033261	Replication protein A	5 Unannotated
STMDT12_C38700	Complement (4033546–4034286)	Integrase	1.4 [L] Replication, recombination, and repair
STMDT12_C38710	Complement (4034656–4034742)	Hypothetical protein	5 Unannotated
STMDT12_C38720	4034805–4035062	Hypothetical protein	5 Unannotated
STMDT12_C38730	Complement (4035018–4035200)	Hypothetical protein	1.3 [K] Transcription
STMDT12_C38740	4035449–4035571	Hypothetical protein	5 Unannotated
STMDT12_C38750	4035732–4036901	Hypothetical protein	5 Unannotated
STMDT12_C38760	Complement (4037496–4037771)	Hypothetical protein	4.1 [R] General function prediction only
STMDT12_C38770	Complement (4037771–4038055)	Hypothetical protein	5 Unannotated
STMDT12_C38780	4038660–4039412	Putative HTH-type transcriptional regulator yfaX	1.3 [K] Transcription
STMDT12_C38790	Complement (4039458–4040423)	2-Keto-3-deoxygluconate permease	5 Unannotated
STMDT12_C38800	Complement (4040456–4040836)	Translation initiation inhibitor	1.1 [J] Translation, ribosomal structure and biogenesis
STMDT12_C38810	Complement (4040861–4041751)	Dihydrodipicolinate synthase	3.3 [E] Amino acid transport and metabolism
STMDT12_C38820	Complement (4041984–4042178)	Hypothetical protein	5 Unannotated
STMDT12_C38830	4042203–4042334	Oxidoreductase	4.1 [R] General function prediction only
STMDT12_C38840	4042365–4042592	Oxidoreductase	4.1 [R] General function prediction only
STMDT12_C38850	4042735–4043601	sn-Glycerol-3-phosphate transport system permease protein ugpA	3.2 [G] Carbohydrate transport and metabolism
STMDT12_C38860	4043591–4044478	l-Arabinose transport system permease protein araQ	3.2 [G] Carbohydrate transport and metabolism
STMDT12_C38870	4044489–4045313	Protein icc	4.1 [R] General function prediction only
STMDT12_C38880	4045319–4046392	sn-Glycerol-3-phosphate transport ATP-binding protein	3.2 [G] Carbohydrate transport and metabolism
STMDT12_C38890	4046385–4047695	Glycerol-3-phosphate-binding periplasmic protein precursor	3.2 [G] Carbohydrate transport and metabolism
STMDT12_C38900	Complement (4047754–4048047)	ISEc13 transposase	5 Unannotated
STMDT12_C38910	Complement (4048139–4048312)	Hypothetical protein	1.4 [L] Replication, recombination, and repair
STMDT12_C38920	Complement (4048394–4048840)	Putative transposase OrfB protein of insertion sequence IS629	1.4 [L] Replication, recombination, and repair
STMDT12_C38930	Complement (4048824–4049282)	Putative transposase OrfB protein of insertion sequence IS629	1.4 [L] Replication, recombination, and repair
STMDT12_C38940	Complement (4049282–4049590)	Putative transposase OrfA protein of insertion sequence IS629	1.4 [L] Replication, recombination, and repair
STMDT12_C38950	4049615–4050319	Transposase InsB4 for insertion sequence IS26	1.4 [L] Replication, recombination, and repair
STMDT12_C38960	Complement (4050353–4050907)	Glucose-1-phosphatase precursor	5 Unannotated
STMDT12_C38970	Complement (4051029–4051514)	Hypothetical protein	5 Unannotated
STMDT12_C38980	Complement (4051539–4052024)	Hypothetical protein	2.7 [O] Posttranslational modification, protein turnover, chaperones
STMDT12_C38990	Complement (4052011–4052706)	Putative ABC transport system ATP-binding component	2.2 [V] Defense mechanisms

Continued on following page

TABLE 1—Continued

Gene_ID	Location (nt positions)	Product	COG category ^a
STMDT12_C39000	Complement (4052711–4053841)	Hypothetical protein	2.2 [V] Defense mechanisms
STMDT12_C39010	Complement (4053831–4055114)	High-affinity Fe ²⁺ binding protein permease component	2.2 [V] Defense mechanisms
STMDT12_C39020	Complement (4055117–4056496)	High-affinity Fe ²⁺ binding protein membrane component	4.2 [S] Function unknown
STMDT12_C39030	Complement (4056600–4057127)	Hypothetical protein	3.7 [P] Inorganic ion transport and metabolism
STMDT12_C39040	Complement (4057168–4059054)	High-affinity Fe ²⁺ /Pb ²⁺ permease	3.7 [P] Inorganic ion transport and metabolism
STMDT12_C39050	Complement (4059401–4060162)	Na ⁺ -translocating NADH-quinone reductase subunit C	3.1 [C] Energy production and conversion
STMDT12_C39060	Complement (4060399–4060905)	Secreted copper sensitivity suppressor D	2.7 [O] Posttranslational modification, protein turnover, chaperones
STMDT12_C39070	Complement (4060895–4061053)	Hypothetical protein	2.7 [O] Posttranslational modification, protein turnover, chaperones
STMDT12_C39080	4061516–4061791	Putative IS1 repressor protein InsA	1.4 [L] Replication, recombination, and repair
STMDT12_C39090	4061710–4062213	Transposase	1.4 [L] Replication, recombination, and repair
STMDT12_C39100	4062380–4062973	Hypothetical protein	1.3 [K] Transcription
STMDT12_C39110	Complement (4063086–4064291)	Tetracycline resistance protein	3.2 [G] Carbohydrate transport and metabolism
STMDT12_C39120	4064373–4064996	Tetracycline repressor protein TetR	1.3 [K] Transcription
STMDT12_C39130	Complement (4064974–4065660)	Hypothetical protein	1.3 [K] Transcription
STMDT12_C39140	Complement (4065668–4066165)	Hypothetical protein	5 Unannotated
STMDT12_C39150	Complement (4066307–4066810)	Insertion element IS1 protein insB	1.4 [L] Replication, recombination, and repair
STMDT12_C39160	Complement (4066729–4067004)	Putative IS1 repressor protein InsA	1.4 [L] Replication, recombination, and repair
STMDT12_C39170	4067283–4067942	Chloramphenicol acetyltransferase	2.2 [V] Defense mechanisms
STMDT12_C39180	Complement (4068143–4068520)	Hypothetical protein	1.3 [K] Transcription
STMDT12_C39190	Complement (4068587–4071553)	Tn3 family transposase	1.4 [L] Replication, recombination, and repair
STMDT12_C39200	Complement (4071556–4072116)	Transposon Tn21 resolvase TnpR	1.4 [L] Replication, recombination, and repair
STMDT12_C39210	Complement (4072242–4072826)	Transposition modulator TnpM	5 Unannotated
STMDT12_C39220	Complement (4072795–4073808)	IntI1	1.4 [L] Replication, recombination, and repair
STMDT12_C39230	4073973–4074848	β-Lactamase OXA-30	2.2 [V] Defense mechanisms
STMDT12_C39240	4074961–4075752	Aminoglycoside resistance protein	4.1 [R] General function prediction only
STMDT12_C39250	4075916–4076263	Multidrug efflux protein	3.7 [P] Inorganic ion transport and metabolism
STMDT12_C39260	4076257–4077096	Dihydropteroate synthase	3.5 [H] Coenzyme transport and metabolism
STMDT12_C39270	Complement (4077026–4077187)	Hypothetical protein	5 Unannotated
STMDT12_C39280	4077224–4077724	Acetyltransferase (GNAT) family protein	1.3 [K] Transcription
STMDT12_C39290	Complement (4077900–4078682)	IstB	1.4 [L] Replication, recombination, and repair
STMDT12_C39300	Complement (4078672–4080195)	Transposase for insertion sequences IS1/326	1.4 [L] Replication, recombination, and repair
STMDT12_C39310	4080318–4081862	InsG	1.4 [L] Replication, recombination, and repair
STMDT12_C39320	Complement (4081913–4082773)	TniB	2.7 [O] Posttranslational modification, protein turnover, chaperones
STMDT12_C39330	Complement (4082776–4084455)	Putative transposase TniA	1.4 [L] Replication, recombination, and repair
STMDT12_C39340	Complement (4084530–4085237)	Urf2	2.3 [T] Signal transduction mechanisms
STMDT12_C39350	Complement (4085234–4085470)	Putative mercury resistance protein	5 Unannotated
STMDT12_C39360	Complement (4085467–4085829)	Transcriptional regulator MerD	1.3 [K] Transcription
STMDT12_C39370	Complement (4085847–4087541)	Putative mercuric reductase	3.7 [P] Inorganic ion transport and metabolism
STMDT12_C39380	Complement (4087593–4088015)	Putative mercury transport protein MerC	5 Unannotated
STMDT12_C39390	Complement (4088051–4088326)	Mercury resistance operon protein MerP	3.7 [P] Inorganic ion transport and metabolism
STMDT12_C39400	Complement (4088340–4088690)	Putative mercuric transport protein	5 Unannotated
STMDT12_C39410	4088762–4089196	Putative transcriptional regulator MerR	1.3 [K] Transcription
STMDT12_C39420	Complement (4089303–4089806)	Insertion element IS1 protein insB	1.4 [L] Replication, recombination, and repair
STMDT12_C39430	Complement (4089725–4090000)	Insertion element IS1 protein	1.4 [L] Replication, recombination, and repair

^a See COG functional category at the website <http://www.ncbi.nlm.nih.gov/COG/old/palox.cgi?fun=all>.

gene sites appear to be associated with sugar-related metabolism, no clear evidence was found to explain this apparent relationship.

GI-DT12 is predicted to be composed of a complex of IS1-related composite transposons, such as class 1 integron, Tn10-like tetracycline resistance determinants, mercury resistance proteins, and iron acquisition systems (aerobactin and an iron transporter). The sequence of GI-DT12 between IS26 and IS1-2c, which accounts for nearly half of the 82-kb GI, is nearly identical to *S. enterica* serovar Typhimurium plasmid pUO-StVR2, suggesting that plasmid DNA is a possible source of this island. The class 1 integron of GI-DT12 and pUO-StVR2 share the *bla*_{OXA-30} gene as a cassette (Fig. 4A), leading to resistance to ampicillin (37) and cefepime (13), whereas the class 1 integron of pSTMDT12_L shares the *dfx1* gene as a

cassette, leading to trimethoprim resistance. It therefore appears that multidrug resistance in T000240 has been acquired in part through IS1- or IS26-related integration on the chromosome and plasmid pSTMDT12_L, respectively (Fig. 4B).

In addition to antibiotic resistance, we determined that heavy metal-related genetic features have been acquired by T000240, with such genes likely contributing to the ability of this strain to adapt to adverse environmental conditions. Indeed, plasmid pRSB107, which was originally isolated from a bacterium inhabiting extremely polluted sewage (39), may represent one possible donor of genetic information. *S. enterica* serovar Typhimurium T000240 possesses a catecholate type of siderophore (enterobactin) synthesis (STMDT12_C06460 to STMDT12_C06580) and has subsequently acquired a citrate-hydroxamate type of siderophore (aerobactin) synthesis on

FIG. 4. Schematic representation of multiple drug resistance determinants. (A) Pairwise comparison of GI-DT12 in T000240 with *S. Typhimurium* plasmid pUO-StVR2 and uncultured bacterium plasmid pRSB107 by a BLASTN homology search and visualized by the ACT program. The red and blue bars between the chromosomal DNA lines shown in gray represent individual nucleotide matches with forward or inverted direction, respectively. BLASTN match scores less than 200 are not shown. (B) Class 1 integron on plasmid pSTMDT12_L. (C) Plasmid pSTMDT12_S.

GI-DT12. The additional acquisition of a distinct siderophore could contribute to iron homeostasis under a free serum iron concentration of $\sim 10^{-24}$ M (29, 32). *S. enterica* serovar Typhimurium strains possess a seroconverting system, consisting of *gtr* orthologs (STMDT12_C06190 to STMDT12_C06201, corresponding to STM0557-0559 in

LT2), in the core genome that is responsible for form variation and glucosylation of the O12 antigen galactose to generate the 12-2 variant (7). The additional seroconverting system present in the prophage (41) was shared in the T000240, DT104, and D23580 strains, but not with LT2 (Fig. 4). Since form variation in *S. enterica* serovar Typhimurium is not constitutive, the

TABLE 2. ORFs in resistance island of pSTMDT12_L

Gene_ID	Location (nt positions)	Product	COG category ^a
STMDT12_L00510	Complement (37106–37810)	IS26 transposase	1.4 [L] Replication, recombination, and repair
STMDT12_L00520	Complement (37857–37979)	Putative transposase	5 Unannotated
STMDT12_L00530	Complement (38097–39233)	Hypothetical protein	5 Unannotated
STMDT12_L00540	Complement (39284–39511)	Hypothetical protein	5 Unannotated
STMDT12_L00550	Complement (40208–40750)	Hypothetical protein	3.4 [F] Nucleotide transport and metabolism
STMDT12_L00560	Complement (40763–41623)	Aminoglycoside 3- <i>N</i> -acetyltransferase	2.2 [V] Defense mechanisms
STMDT12_L00570	41856–42560	IS26 transposase	1.4 [L] Replication, recombination, and repair
STMDT12_L00580	Complement (42612–42725)	Hypothetical protein	1.3 [K] Transcription
STMDT12_L00590	Complement (42736–43941)	Chromate transport protein	3.7 [P] Inorganic ion transport and metabolism
STMDT12_L00600	Complement (44097–44300)	Hypothetical protein	5 Unannotated
STMDT12_L00610	44337–44498	Hypothetical protein	5 Unannotated
STMDT12_L00620	Complement (44428–45267)	Dihydropteroate synthase	3.5 [H] Coenzyme transport and metabolism
STMDT12_L00630	Complement (45261–45608)	Multidrug efflux protein	3.7 [P] Inorganic ion transport and metabolism
STMDT12_L00640	Complement (45772–46563)	Aminoglycoside resistance protein	4.1 [R] General function prediction only
STMDT12_L00650	Complement (46569–46859)	Hypothetical protein	5 Unannotated
STMDT12_L00660	Complement (46971–47468)	Dihydrofolate reductase	3.5 [H] Coenzyme transport and metabolism
STMDT12_L00670	47613–48626	Int11	1.4 [L] Replication, recombination, and repair
STMDT12_L00680	48595–48867	Transposon Tn21 modulator protein	2.2 [V] Defense mechanisms
STMDT12_L00690	Complement (48901–49605)	IS26 transposase	1.4 [L] Replication, recombination, and repair

^a See COG functional category at the website <http://www.ncbi.nlm.nih.gov/COG/old/palox.egi?fun=all>.

redundant seroconverting systems might be involved in intestinal persistence, fecal shedding, and transmission (7).

In conclusion, the present study represents the first characterization of a unique genomic island, GI-DT12, that is associated with possible IS1-mediated recombination in *S. enterica* serovar Typhimurium. Such ISSs may represent potential landmarks to obtain a variety of genetic elements present on the plasmids and chromosomes of pathogenic bacteria. It is expected that further whole-genome studies will aid in the characterization of the horizontal gene transfer events responsible for the antibiotic resistance and virulence of emerging *S. enterica* serovar Typhimurium strains.

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REFERENCES

- Aarestrup, F. M., et al. 2007. International spread of multidrug-resistant *Salmonella* Schwarzengrund in food products. *Emerg. Infect. Dis.* **13**:726–731.
- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**:403–410.
- Anderson, E. S., L. R. Ward, M. J. Saxe, and J. D. de Sa. 1977. Bacteriophage-typing designations of *Salmonella typhimurium*. *J. Hyg. (Lond.)* **78**:297–300.
- Angulo, F. J., V. N. Nargund, and T. C. Chiller. 2004. Evidence of an association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans and the human health consequences of such resistance. *J. Vet. Med. B Infect. Dis. Vet. Public Health* **51**:374–379.
- Bao, H., et al. 2009. MapView: visualization of short reads alignment on a desktop computer. *Bioinformatics* **25**:1554–1555.
- Besemer, J., A. Lomsadze, and M. Borodovsky. 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes: implications for finding sequence motifs in regulatory regions. *Nucleic Acids Res.* **29**:2607–2618.
- Bogomolnaya, L. M., C. A. Santiviago, H. J. Yang, A. J. Baumler, and H. L. Andrews-Polymenis. 2008. 'Form variation' of the O12 antigen is critical for persistence of *Salmonella* Typhimurium in the murine intestine. *Mol. Microbiol.* **70**:1105–1119.
- Carver, T., et al. 2008. Artemis and ACT: viewing, annotating and comparing sequences stored in a relational database. *Bioinformatics* **24**:2672–2676.
- Chaisson, M. J., and P. A. Pevzner. 2008. Short read fragment assembly of bacterial genomes. *Genome Res.* **18**:324–330.
- Chen, C. Y., G. W. Nace, B. Solow, and P. Fratamico. 2007. Complete nucleotide sequences of 84.5- and 3.2-kb plasmids in the multi-antibiotic resistant *Salmonella enterica* serovar Typhimurium U302 strain G8430. *Plasmid* **57**:29–43.
- de Lorenzo, V., M. Herrero, and J. B. Neilands. 1988. IS1-mediated mobility of the aerobactin system of pColV-K30 in *Escherichia coli*. *Mol. Gen. Genet.* **213**:487–490.
- de Lorenzo, V., and J. B. Neilands. 1986. Characterization of *iucA* and *iucC* genes of the aerobactin system of plasmid ColV-K30 in *Escherichia coli*. *J. Bacteriol.* **167**:350–355.
- Dubois, V., et al. 2003. Decreased susceptibility to cefepime in a clinical strain of *Escherichia coli* related to plasmid- and integron-encoded OXA-30 beta-lactamase. *Antimicrob. Agents Chemother.* **47**:2380–2381.
- Dutil, L., et al. 2010. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. *Emerg. Infect. Dis.* **16**:48–54.
- Fricke, W. F., et al. 2009. Antimicrobial resistance-conferring plasmids with similarity to virulence plasmids from avian pathogenic *Escherichia coli* strains in *Salmonella enterica* serovar Kentucky isolates from poultry. *Appl. Environ. Microbiol.* **75**:5963–5971.
- Gilks, C. F., et al. 1990. Life-threatening bacteraemia in HIV-1 seropositive adults admitted to hospital in Nairobi, Kenya. *Lancet* **336**:545–549.
- Golebiewski, M., et al. 2007. Complete nucleotide sequence of the pCTX-M3 plasmid and its involvement in spread of the extended-spectrum beta-lactamase gene blaCTX-M-3. *Antimicrob. Agents Chemother.* **51**:3789–3795.
- Gordon, M. A., et al. 2008. Epidemics of invasive *Salmonella enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin. Infect. Dis.* **46**:963–969.
- Guerry, P., J. van Embden, and S. Falkow. 1974. Molecular nature of two nonconjugative plasmids carrying drug resistance genes. *J. Bacteriol.* **117**:619–630.
- Herrero, A., M. C. Mendoza, R. Rodicio, and M. R. Rodicio. 2008. Characterization of pUO-StVR2, a virulence-resistance plasmid evolved from the pSLT virulence plasmid of *Salmonella enterica* serovar Typhimurium. *Antimicrob. Agents Chemother.* **52**:4514–4517.
- Herrero, A., M. R. Rodicio, M. A. Echeita, and M. C. Mendoza. 2008. *Salmonella enterica* serotype Typhimurium carrying hybrid virulence-resistance plasmids (pUO-StVR): a new multidrug-resistant group endemic in Spain. *Int. J. Med. Microbiol.* **298**:253–261.
- Holt, K. E., et al. 2007. Multidrug-resistant *Salmonella enterica* serovar Paratyphi A harbors IncHI1 plasmids similar to those found in serovar typhi. *J. Bacteriol.* **189**:4257–4264.
- Hsu, W. B., and J. H. Chen. 2003. The IS1 elements in *Shigella boydii*: horizontal transfer, vertical inactivation and target duplication. *FEMS Microbiol. Lett.* **222**:289–295.
- Izumiya, H., et al. 2005. Characterization of isolates of *Salmonella enterica* serovar typhimurium displaying high-level fluoroquinolone resistance in Japan. *J. Clin. Microbiol.* **43**:5074–5079.
- Johnson, T. J., and L. K. Nolan. 2009. Pathogenomics of the virulence plasmids of *Escherichia coli*. *Microbiol. Mol. Biol. Rev.* **73**:750–774.
- Li, H., J. Ruan, and R. Durbin. 2008. Mapping short DNA sequencing reads and calling variants using mapping quality scores. *Genome Res.* **18**:1851–1858.
- McClelland, M., et al. 2001. Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. *Nature* **413**:852–856.
- McEwen, S. A., and P. J. Fedorka-Cray. 2002. Antimicrobial use and resistance in animals. *Clin. Infect. Dis.* **34**(Suppl. 3):S93–S106.
- Miethke, M., and M. A. Marahiel. 2007. Siderophore-based iron acquisition and pathogen control. *Microbiol. Mol. Biol. Rev.* **71**:413–451.
- Nakaya, H., et al. 2003. Life-threatening infantile diarrhea from fluoroquinolone-resistant *Salmonella enterica* typhimurium with mutations in both *gyrA* and *parC*. *Emerg. Infect. Dis.* **9**:255–257.
- Rabsch, W., et al. 2002. *Salmonella enterica* serotype Typhimurium and its host-adapted variants. *Infect. Immun.* **70**:2249–2255.
- Raymond, K. N., E. A. Dertz, and S. S. Kim. 2003. Enterobactin: an archetype for microbial iron transport. *Proc. Natl. Acad. Sci. U. S. A.* **100**:3584–3588.
- Richter, D. C., S. C. Schuster, and D. H. Huson. 2007. OSLay: optimal syntenic layout of unfinished assemblies. *Bioinformatics* **23**:1573–1579.
- Runyen-Janecky, L. J., S. A. Reeves, E. G. Gonzales, and S. M. Payne. 2003. Contribution of the *Shigella flexneri* Sit, Iuc, and Feo iron acquisition systems to iron acquisition in vitro and in cultured cells. *Infect. Immun.* **71**:1919–1928.
- Saedler, H., and B. Heiss. 1973. Multiple copies of the insertion-DNA sequences IS1 and IS2 in the chromosome of *Escherichia coli* K-12. *Mol. Gen. Genet.* **122**:267–277.
- Scholz, P., et al. 1989. Complete nucleotide sequence and gene organization of the broad-host-range plasmid RSF1010. *Gene* **75**:271–288.
- Siu, L. K., et al. 2000. beta-lactamases in *Shigella flexneri* isolates from Hong Kong and Shanghai and a novel OXA-1-like beta-lactamase, OXA-30. *Antimicrob. Agents Chemother.* **44**:2034–2038.
- Stamatakis, A., T. Ludwig, and H. Meier. 2005. RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics* **21**:456–463.
- Szczepanowski, R., et al. 2005. The 120 592-bp IncF plasmid pRSB107 isolated from a sewage-treatment plant encodes nine different antibiotic-resistance determinants, two iron-acquisition systems and other putative virulence-associated functions. *Microbiology* **151**:1095–1111.
- Tanaka, K., et al. 2004. Molecular characterization of a prophage of *Salmonella enterica* serotype Typhimurium DT104. *J. Clin. Microbiol.* **42**:1807–1812.
- Vander Byl, C., and A. M. Kropinski. 2000. Sequence of the genome of *Salmonella* bacteriophage P22. *J. Bacteriol.* **182**:6472–6481.
- Yau, S., X. Liu, S. P. Djordjevic, and R. M. Hall. 2010. RSF1010-like plasmids in Australian *Salmonella enterica* serovar Typhimurium and origin of their *sul2-strA-strB* antibiotic resistance gene cluster. *Microb. Drug Resist.* **16**:249–252.
- Zhang, S., et al. 2003. Molecular pathogenesis of *Salmonella enterica* serotype Typhimurium-induced diarrhea. *Infect. Immun.* **71**:1–12.
- Zhao, S., et al. 2003. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. *J. Clin. Microbiol.* **41**:5366–5371.