Applied and Environmental Microbiology, Aug. 2011, p. 5655–5664 Vol. 77, No. 16
0099-2240/11/$12.00 doi:10.1128/AEM.00425-11

Antimicrobial Resistance and Virulence Determinants in European *Salmonella* Genomic Island 1-Positive *Salmonella enterica* Isolates from Different Origins▽†

Janine Beutlich,[1] Silke Jahn,[1] Burkhard Malorny,[1] Elisabeth Hauser,[1] Stephan Hühn,[2] Andreas Schroeter,[1] Maria Rosario Rodicio,[3] Bernd Appel,[1] John Threlfall,[4] Dik Mevius,[5] Reiner Helmuth,[1] and Beatriz Guerra,[1]* on behalf of the Med-Vet-Net WP21 Project Group

Federal Institute for Risk Assessment, Department of Biological Safety, Thielallee 88-92, D-14195 Berlin, Germany[1]; Free University Berlin, Institute of Food Hygiene, Königsweg 69, D-14163 Berlin, Germany[2]; University of Oviedo, Department of Functional Biology, Area of Microbiology, C/Julián Clavería 6, E-33006 Oviedo, Asturias, Spain[3]; Health Protection Agency, Centre for Infections, 61 Colindale Avenue, London NW9 5EQ, United Kingdom[4]; and Central Veterinary Institute, Department of Bacteriology and TSEs, P.O. Box 2004, NL-8203 AB Lelystad, The Netherlands[5]

Received 25 February 2011/Accepted 6 June 2011

***Salmonella* genomic island 1 (SGI1) contains a multidrug resistance region conferring the ampicillin-chloramphenicol-streptomycin-sulfamethoxazole-tetracycline resistance phenotype encoded by bla_{PSE-1}, *floR*, *aadA2*, *sul1*, and *tet*(G). Its increasing spread via interbacterial transfer and the emergence of new variants are important public health concerns. We investigated the molecular properties of SGI1-carrying *Salmonella enterica* serovars selected from a European strain collection. A total of 38 strains belonging to *S. enterica* serovar Agona, *S. enterica* serovar Albany, *S. enterica* serovar Derby, *S. enterica* serovar Kentucky, *S. enterica* serovar Newport, *S. enterica* serovar Paratyphi B dT+, and *S. enterica* serovar Typhimurium, isolated between 2002 and 2006 in eight European countries from humans, animals, and food, were subjected to antimicrobial susceptibility testing, molecular typing methods (XbaI pulsed-field gel electrophoresis [PFGE], plasmid analysis, and multilocus variable-number tandem-repeat analysis [MLVA]), as well as detection of resistance and virulence determinants (PCR/sequencing and DNA microarray analysis). Typing experiments revealed wide heterogeneity inside the strain collection and even within serovars. PFGE analysis distinguished a total of 26 different patterns. In contrast, the characterization of the phenotypic and genotypic antimicrobial resistance revealed serovar-specific features. Apart from the classical SGI1 organization found in 61% of the strains, seven different variants were identified with antimicrobial resistance properties associated with SGI1-A (*S.* Derby), SGI1-C (*S.* Derby), SGI1-F (*S.* Albany), SGI1-L (*S.* Newport), SGI1-K (*S.* Kentucky), SGI1-M (*S.* Typhimurium), and, eventually, a novel variant similar to SGI1-C with additional gentamicin resistance encoded by *aadB*. Only minor serovar-specific differences among virulence patterns were detected. In conclusion, the SGI1 carriers exhibited pathogenetic backgrounds comparable to the ones published for susceptible isolates. However, because of their multidrug resistance, they may be more relevant in clinical settings.**

Salmonella genomic island 1 (SGI1) is a 43-kb mobilizable chromosomal region that is located in *Salmonella enterica* serovars between the genes *thdF* and *yidY* or between *thdF* and *int2* in the retron sequence of *S. enterica* serovar Typhimurium (7). In 2008, an SGI1 secondary chromosomal attachment site for integration of the island between the *sodB* and *purR* genes was described (17). SGI1 contains an antimicrobial resistance gene cluster (7, 16) located on a 13-kb complex class 1 integron designated In104 (31), which confers pentaresistance (Penta-R) to ampicillin (bla_{PSE-1}), chloramphenicol/florfenicol (*floR*), streptomycin/spectinomycin (*aadA2*), sulfamethoxazole (*sul1*), and tetracycline [*tet*(G)] (8). The genes bla_{PSE-1} and *aadA2* are located on class 1 integrons, named InC and InD, with variable regions of 1.2 kb and 1.0 kb, respectively (3, 24). The five resistance determinants are characteristic of the pandemic *S.* Typhimurium definite phage type 104 (DT104), which was globally most predominant in causing infections in humans since the 1980s (16, 21, 38). SGI1 was first identified in 2000 in a Canadian multidrug-resistant *S.* Typhimurium DT104 strain with the Penta-R phenotype (7). Subsequently SGI1 and several variant SGI1 antimicrobial resistance gene clusters were detected worldwide in different *Salmonella* serovars and in *Proteus mirabilis* (1, 2, 15, 31, 39, 40).

Within the European Union (EU) Network of Excellence Med-Vet-Net, an investigation on the molecular epidemiology of SGI1 in enteric bacteria other than *S.* Typhimurium DT104 was conducted (http://www.medvetnet.org). For this study, a collection of 445 multidrug-resistant strains (*S. enterica*, 277; *Escherichia coli*, 116; *Shigella* spp., 43, and *Proteus* spp., 9), isolated from animals, food, and humans from eight European

* Corresponding author. Mailing address: Federal Institute for Risk Assessment (BfR), Thielallee 88-92, D-14195 Berlin, Germany. Phone: 49-30-8412-2082. Fax: 49-30-8412-2953. E-mail: beatriz.guerra@bfr.bund.de.

† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 24 June 2011.

countries between the years 2002 and 2006, was investigated (3). By conventional gel-based PCR assays, 53% of the *Salmonella* isolates were SGI1 positive, showing fragments of both typical left and right SGI1 junctions (3, 8). Application of real-time PCR targeting open reading frame (ORF) S004 of SGI1 detected the island in five additional *Salmonella* strains. These strains lacked both SGI1 junctions (one *S. enterica* serovar Agona and three *S. enterica* serovar Derby) or showed the left junction only (one *S.* Typhimurium) (3).

The spread of multidrug resistance (MDR) among bacterial populations is alarming, and the detection of SGI1 is regarded as important for public health (3). Therefore, the characterization of SGI1-carrying bacteria as presented in this study is a useful tool to support efforts in the health care sector. Taking this into account, we selected from the collection of Amar et al. (3) all strains that acquired SGI1 and belonged to serovars other than *S.* Typhimurium or showed phage types unrelated to DT104. In order to gain more knowledge on their phenotypic and molecular backgrounds, all isolates were analyzed for their antimicrobial resistance phenotypes and the underlying molecular mechanisms, screened for selected virulence genes, and subjected to molecular typing. In this way, a well-characterized collection of strains was set up to be used as controls for several molecular-epidemiological approaches to antimicrobial resistance and virulence.

MATERIALS AND METHODS

Participating institutes. Institutes from eight European countries provided SGI1-positive *Salmonella enterica* subsp. *enterica* isolates for this study: Agence Nationale de Sécurité Sanitaire de L'alimentation de L'environment et du Travail (ANSES), Maisons-Alfort, France; Central Veterinary Institute (CVI), Lelystad, The Netherlands; Federal Institute for Risk Assessment (BfR), Berlin, Germany; Istituto Superiore di Sanità (ISS), Rome, Italy; Statens Serum Institute (SSI), Copenhagen, Denmark; Panstwowy Zaklad Higieny (PZH), Warsaw, Poland; Veterinary Laboratories Agency (VLA), Weybridge, United Kingdom; and Veterinary Medical Research Institute (VMRI), Budapest, Hungary.

Bacterial isolates. In a previous work, the Health Protection Agency (HPA), Laboratory of Enteric Pathogens, United Kingdom, analyzed between May 2006 and March 2007 a European selection of 445 bacterial isolates for the presence of SGI1. In this study, a total of 152 *Salmonella* isolates tested positive for SGI1 (3). Of these, 38 strains were sent to the BfR, Berlin, Germany, for further molecular analyses. This collection of SGI1-positive isolates included (i) all isolates belonging to serovars other than *S.* Typhimurium, i.e., *S.* Agona (1 isolate), *S. enterica* serovar Kentucky (1), *S. enterica* serovar Paratyphi B dT+ (*S. enterica* serovar Java) (1), *S. enterica* serovar Albany (3), *S. enterica* serovar Newport (4), and *S.* Derby (5); (ii) *S.* Typhimurium non-DT104 (18); and, for comparison, (iii) *S.* Typhimurium DT104 (5). The strains were isolated between 2002 and 2006 from humans (19 isolates), animals (10), and food (9) in the eight European countries (Table 1). We included in this series all isolates (15) that showed atypical SGI1 properties (lack of SGI1 markers) in the collection described by Amar et al. (3). These 15 isolates were designated "unconventional" in the present study (Table 2).

Phenotypic analysis. Serotyping was done following the White-Kauffmann-Le Minor scheme (22). Phage typing of *S.* Typhimurium isolates was performed according to the method of Anderson et al. (4). All isolates were tested by broth microdilution, according to the guidelines of the CLSI (11), for their susceptibility to 17 antimicrobial agents: ampicillin (AMP), amoxicillin-clavulanic acid (AMC), ceftiofur (XNL), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), florfenicol (FLO), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), neomycin (NEO), spectinomycin (SPE), streptomycin (STR), sulfamethoxazole (SUL), tetracycline (TET), trimethoprim (TMP), and sulfamethoxazole/trimethoprim (SXT), using custom-defined microtiter plates (Trek Diagnostic Systems, United Kingdom). The antimicrobial concentrations tested were described by Schroeter et al. (37). The breakpoints used are shown in Table 1.

Molecular typing. Plasmid profiling was performed by alkaline denaturation according to the method of Kado and Liu (28). Vertical electrophoresis was carried out on 0.8% agarose (standard low melting point; Bio-Rad Laboratories, Munich, Germany) gels for 3 to 4 h at 100 V. The *E. coli* reference plasmids R27 (169 kb), R1 (94 kb), RP4 (55 kb), and ColE1 (6 kb) were used as standards for the determination of plasmid sizes. Genomic DNA from the SGI1 isolates was subjected to macrorestriction analysis with the XbaI endonuclease (Roche Diagnostics, Mannheim, Germany). The generated fragments were separated by pulsed-field gel electrophoresis (PFGE) using the CHEF-DRIII SYS220/240 system (Bio-Rad Laboratories, Munich, Germany). Agarose plug preparation and PFGE running conditions followed the PulseNet standardized protocol (http://www.pulsenet-europe.org). The resulting profiles were analyzed by recording the presence or absence of fragments larger than 33 kb. Profiles with differences in two or more bands were designated using numbers (e.g., X1, X2, etc.). Similar patterns in which only one band, or rather, the intensity of a band, was different were designated using lowercase letters (e.g., X3 and X3a). Multilocus variable-number tandem-repeat analysis (MLVA) of *S.* Typhimurium isolates was performed as described by Lindstedt et al. (32) using an ABI 310 DNA Sequencer (Applied Biosystems, Darmstadt, Germany). MLVA nomenclature according to Larsson et al. (29) was used.

Detection of resistance and virulence determinants. In a first approach, detection of resistance and virulence genes in all isolates was undertaken by PCR amplification (primers and conditions are given in Table SA in the supplemental material). The isolates were analyzed for the presence of resistance genes related to their resistance phenotypes. Overall, genes encoding resistance to ampicillin (bla_{PSE1}, $bla_{TEM1\text{-}like}$, and $bla_{OXA1\text{-}like}$), chloramphenicol (*catA1*, *cmlA*, and *floR*), gentamicin [*aacC2*, *aacC4*, *aac(3)-Ie* (*aacC5*), *aadB*, and *armA*], kanamycin [*aphA1*, *aphA2*, and *aac(6)-1b*], fluoroquinolones (*qnrA*, *qnrB*, and *qnrS*), streptomycin (*aadA1*-like, *aadA2*, *aadA7*, *and strA-strB*), sulfamethoxazole (*sul1*, *sul2*, and *sul3*), tetracycline [*tet*(A), *tet*(B), and *tet*(G)], and trimethoprim (*dfrA1*-like, *dfrA5-14*, *dfrA7-17*, *dfrA10*, *dfrA12*, and *dfrA17*) were screened as previously described (23, 35). The presence of class 1 and 2 integrons was investigated by using the 5′-CS/3′-CS and Hep74/Hep51 primers to amplify their variable regions (30, 41). Gene cassettes inserted therein were identified by DNA sequencing of the amplicons performed by Agowa GmbH (Berlin, Germany). The sequences obtained were compared using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

For virulotyping analysis, isolates were screened for the presence of 10 virulence genes (*avrA*, *bcfC*, *gipA*, *mgtC*, *ssaQ*, *sopB*, *sopC1*, *sopE1*, *spvC*, and *spi4_D*). PCR amplifications were performed as described by Huehn et al. (27).

In a second approach, nine isolates (4 *S.* Typhimurium, 1 *S.* Newport, 1 *S.* Paratyphi B dT+, 1 *S.* Albany, 1 *S.* Kentucky, and 1 *S.* Derby), representing different serovars, phage types, countries, and/or origins, were selected for an extensive analysis of the presence of virulence determinants using DNA microarrays (see Fig. 2). Probes, microarray production, and performance of whole-genome DNA labeling, hybridization, analysis, and validation were as previously described (26). Virulence determinants for each strain analyzed (101 genes) were categorized according to their locations on the *Salmonella* genome: SPIs, prophages, plasmids, islets, and fimbrial clusters. Signals that were assigned as "uncertain" (cutoff between 0.25 and 0.4) by microarray analysis were verified by PCR as described previously (26), and an individual decision was made for the presence or absence of each target.

Additional analysis of unconventional isolates lacking a classical chromosomal attachment site. Mapping of the regions flanking the primary chromosomal integration site of SGI1 was performed for a selected group of five isolates already described as unconventional by Amar et al. (3): *S.* Agona 08-02864 (MDR region and S004 present; other markers outside the MDR region and left and right junctions absent); *S.* Derby 08-02866, 08-02867, and 08-02868 (only S004 present); and *S.* Typhimurium 08-02865 (right junction absent). For this, PCR amplifications were carried out targeting genes framing the SGI1 region: upstream (*dnaN*, *dnaA*, *rpmH*, *rnpA*, *yidC*, and *thdF*) and downstream (*int2*, *urt*, *rt*, *yid*Y, *yidZ*, *yieE*, *yieF*, *yieG*, and *yieH*). The primers for the genes cited above were designed from the sequence of *S.* Typhimurium LT2 (accession number AE008879) (see Table SA in the supplemental material).

To detect a secondary integration site of SGI1, previously reported in the intergenic region between the chromosomal genes *sodB* and *purR*, primers sodB-F and purR-B were used (17). Only in case of a nonintegration of SGI1 was an amplicon size of 508 bp expected.

Data analysis. Results were analyzed with BioNumerics software (version 5.1; Applied Maths, Sint-Martens-Latem, Belgium).

TABLE 1. Molecular typing of *Salmonella* isolates used in the study

Country[a]	NRL-Salm no. (isolation yr)	Serotype	Origin	Phage type	Resistance phenotype[b]	PFGE profile	Plasmid size(s) (kb)	MLVA type (STTR 9-5-6-10-3)
NL	08-02864 (04)	*S.* Agona	Human	ND[h]	[Penta][c]	XAg1	≤6	ND
D	08-00310 (02)	*S.* Albany	Fungi	ND	AMP/AMC-CHL/FLO-STR(i)[d]-SUL-TET-TMP/SXT-NAL	XAl1	≤6	ND
F	08-00265 (06)	*S.* Albany	Food	ND	AMP/AMC-CHL/FLO-STR(i)[d]-SUL-TET-TMP/SXT-NAL	XAl2	≤6	ND
F	08-00309 (06)	*S.* Albany	Food	ND	AMP/AMC-CHL/FLO-STR(i)[d]-SUL-TET-TMP/SXT-NAL	XAl2	≤6	ND
NL	08-00263 (04)	*S.* Derby	Human	ND	[Penta]-TMP/SXT-NAL	XD1	110	ND
NL	08-00311 (04)	*S.* Derby	Human	ND	[Penta]-TMP/SXT	XD2	None	ND
NL	08-02866 (05)	*S.* Derby	Human	ND	STR/SPE-SUL-TET	XD3	90, 30	ND
NL	08-02867 (05)	*S.* Derby	Human	ND	STR/SPE-SUL-TET	XD3a	15, ≤6	ND
NL	08-02868 (05)	*S.* Derby	Human	ND	STR/SPE-SUL-TET	XD3a	30, 8	ND
UK	08-00267 (04)	*S.* Paratyphi B dT+	Cattle	ND	[Penta]	XJ1	110	ND
PL	08-00266 (03)	*S.* Kentucky	Human	ND	AMP/AMC-STR/SPE-SUL-TET-GEN-NAL	XK1	None	ND
DK	08-00268 (05)	*S.* Newport	Human	ND	[Penta]-GEN-KAN/NEO-TMP/SXT-NAL	XN1	None	ND
DK	08-00269 (05)	*S.* Newport	Human	ND	[Penta]-GEN-KAN/NEO-TMP/SXT-NAL	XN2	None	ND
DK	08-00313 (05)	*S.* Newport	Human	ND	[Penta]-GEN-TMP/SXT-NAL	XN3	≤6	ND
DK	08-00314 (05)	*S.* Newport	Human	ND	[Penta]-GEN-KAN/NEO-TMP/SXT-NAL	XN3	None	ND
D	08-00325 (04)	*S.* Typhimurium	Sausage	DT193	[Penta]	XT1	90, 10, 7, ≤6	3-14-15-21-311
D	08-00326 (05)	*S.* Typhimurium	Swine	RDNC[e]	[Penta]-TMP/SXT	XT2a	90, 100, 9, 7, ≤6	3-14-15-13-311
D	08-00272 (05)	*S.* Typhimurium	Swine	206	[Penta]-GEN-TMP/SXT	XT2	90, 100, 150	3-14-13-14-311
D	08-02885 (05)	*S.* Typhimurium	Rabbit	DT104L	GEN-STR/SPE-SUL	XT3	90, ≤6	3-13-8-24-311
D	08-02886 (05)	*S.* Typhimurium	Rodent	DT120	AMP/AMC-CHL/FLO-SUL-TET-GEN-TMP/SXT	XT3	90, 55, ≤6, ≤6	3-13-8-24-311
NL	08-00264 (05)	*S.* Typhimurium	Chicken	DT104L	[Penta]-NAL	XT3	90, 7, ≤ 6	3-12-10-24-311
NL	08-02865 (05)	*S.* Typhimurium	Human	DT104B low	[Penta]-GEN	XT3	90, 20, ≤6	3-14-13-18-311
NL	08-00315 (05)	*S.* Typhimurium	Rapeseed	DT104L	[Penta]	XT7	90, 180	3-16-17-22-311
NL	08-00312 (05)	*S.* Typhimurium	Human	DT104L	[Penta]	XT8	90, ≤6	3-11-18-17-311
NL	08-00316 (04)	*S.* Typhimurium	Human	NT[f]	[Penta]-NAL(i)	XT3	90, ≤6, ≤6, ≤6	3-17-6-23-311
NL	08-00270 (04)	*S.* Typhimurium	Pig	U309	[Penta]-TMP/SXT	XT4	90, 50, ≤6	3-15-12-15-311
NL	08-00317 (05)	*S.* Typhimurium	Pig	U309	[Penta]	XT9	130, 90, 9, 7, ≤6	3-10-14-26-311
NL	08-00318 (05)	*S.* Typhimurium	Human	206	[Penta]-GEN	XT2	90, 150	3-14-14-13-311
NL	08-00319 (05)	*S.* Typhimurium	Pig	RDNC	[Penta]	XT2a	90, 9, 7, ≤6	3-14-16-23-311
I	08-00322 (04)	*S.* Typhimurium	Human	NT	[Penta]-TMP/SXT	XT3	90, 25, ≤6, ≤6	3-10-17-14-311
I	08-00271 (04)	*S.* Typhimurium	Human	U309	[Penta]-KAN/NEO-NAL	XT3a	90	3-14-16-21-311
DK	08-00323 (05)	*S.* Typhimurium	Human	NT	[Penta]-GEN	XT5	95, 90, ≤6	3-15-14-16-311
DK	08-00324 (05)	*S.* Typhimurium	Human	DT92	[Penta]-GEN	XT6	170, 90	3-14-15-14-311
UK	08-00320 (04)	*S.* Typhimurium	Sheep	DT193	[Penta]	XT10	90	3-14-12-24-311
UK	08-00321 (05)	*S.* Typhimurium	Cattle	DT151	[Penta]	XT2a	90, ≤6	3-14-16-13-311
H	08-00327 (06)	*S.* Typhimurium	Goose	RDNC	[Penta]	XT11	90, 45, 30, 10, ≤6	3-11-5-NA[g]-311
H	08-00328 (06)	*S.* Typhimurium	Goose	RDNC	[Penta]	XT11	90, 30, ≤6	3-11-5-NA-311
H	08-00329 (06)	*S.* Typhimurium	Pork sausage	RDNC	[Penta]-GEN-TMP/SXT	XT2b	90, 100, 150	3-14-14-14-311

[a] Country abbreviations: D, Germany; DK, Denmark; F, France; H, Hungary; I, Italy; NL, Netherlands; PL, Poland; UK, United Kingdom.

[b] For abbreviations, see Materials and Methods. Resistance phenotypes were assessed by the broth microdilution method, as described by Schroeter et al. (37), by following the guidelines of the CLSI (11). The breakpoints used for AMP, AMC, XNL, CHL, CIP, GEN, KAN, SUL, and TET were the CLSI breakpoints (12). For CIP, the MIC breakpoints were as follows: resistant, ≥4 μg/ml; intermediate, 2 μg/ml; and susceptible, ≤1 μg/ml. The breakpoints used for COL (resistant, ≥16 μg/ml; susceptible, ≤8 μg/ml), FLO (resistant, ≥32 μg/ml; susceptible, ≤8 μg/ml), NAL (resistant, ≥32 μg/ml; susceptible, ≤16 μg/ml), SPE (resistant, ≥128 μg/ml; susceptible, ≤64 μg/ml), and TMP (resistant, ≥16 μg/ml; susceptible, ≤8 μg/ml) were the DANMAP breakpoints (19). Finally, the breakpoints used for NEO (resistant, ≥16 μg/ml; susceptible, ≤4 μg/ml), STR (resistant, ≥32 μg/ml; susceptible, ≤8 μg/ml), and SXT (resistant, ≥4 and ≥76 μg/ml respectively; susceptible, ≤2 and ≤38 μg/ml, respectively) were taken from Schroeter et al. (37).

[c] [Penta], SGI-1 characteristic pentaresistance phenotype [AMP/AMC-CHL/FLO-STR/SPE-SUL-TET].

[d] Intermediate (i) resistance phenotype to STR (MIC, 16 μg/ml) (37).

[e] RDNC, reacts with phages but does not conform to definite or provisional types.

[f] NT, nontypeable.

[g] NA, locus not present.

[h] ND, not done.

TABLE 2. Detection of SGI1 and virulence and resistance determinants

Country[a]	NRL-Salm no. (isolation yr)	Serotype	Origin	SGI1[b]	Resistance phenotype[c]	Resistance genotype	Class 1 integron	Virulence genotype
NL	08-02864 (04)	*S.* Agona (U)[m]	Human	S004	[Penta][d]	[Penta-R][e]	[I][f]	[V-core][g]-*avrA*
D	08-00310 (02)	*S.* Albany (U)	Fungi	LJ/RJ	AMP/AMC-CHL/FLO-STR(i)[h]-SUL-TET-TMP/SXT-NAL	*bla*$_{PSE-1}$-*floR-sul1-tet*(G)-*dfrA1-gyrA*$_{Asp87\rightarrow Asn87}$	1,200 bp/*bla*$_{PSE-1}$ + 1,250 bp/*dfrA1-orf*	[V-core]-*avrA*
F	08-00265 (06)	*S.* Albany (U)	Food	LJ/RJ	AMP/AMC-CHL/FLO-STR(i)-SUL-TET-TMP/SXT-NAL	*bla*$_{PSE-1}$-*floR-sul1-tet*(G)-*dfrA1-gyrA*$_{Asp87\rightarrow Asn87}$	1,200 bp/*bla*$_{PSE-1}$ + 1,250 bp/*dfrA1-orf*	[V-core]-*avrA*
F	08-00309 (06)	*S.* Albany (U)	Food	LJ/RJ	AMP/AMC-CHL/FLO-STR(i)-SUL-TET-TMP/SXT-NAL	*bla*$_{PSE-1}$-*floR-sul1-tet*(G)-*dfrA1-gyrA*$_{Asp87\rightarrow Asn87}$	1,200 bp/*bla*$_{PSE-1}$ + 1,250 bp/*dfrA1-orf*	[V-core]-*avrA*
NL	08-00263 (04)	*S.* Derby	Human	LJ/RJ	[Penta] -TMP/SXT-NAL	[Penta-R]-*dfrA10-gyrA*$_{Ser83\rightarrow Ala83}$	[I]	[V-core]-*avrA*
NL	08-00311 (04)	*S.* Derby	Human	LJ/RJ	[Penta] -TMP/SXT	[Penta-R]-*dfrA10-gyrA*$_{Ser83\rightarrow Ala83}$	[I]	[V-core]-*avrA*
NL	08-02866 (05)	*S.* Derby (U)	Human	S004	STR/SPE-SUL-TET	*aadA2-sul1-tet*(A)	1,000 bp/*aadA2*	[V-core]-*avrA-sopE1*
NL	08-02867 (05)	*S.* Derby (U)	Human	S004	STR/SPE-SUL-TET	*aadA2-sul1-tet*(A)	1,000 bp/*aadA2*	[V-core]-*avrA-sopE1*
NL	08-02868 (05)	*S.* Derby (U)	Human	S004	STR/SPE-SUL-TET	*aadA2-sul1-tet*(A)	1,000 bp/*aadA2*	[V-core]-*avrA-sopE1*
UK	08-00267 (04)	*S.* Paratyphi B dT+	Cattle	LJ/RJ	[Penta]	[Penta-R]-*sul2*	[I]	[V-core]-*sodC1*
PL	08-00266 (03)	*S.* Kentucky (U)	Human	LJ/RJ	AMP/AMC-STR/SPE-SUL-TET-GEN-NAL	*bla*$_{TEM-1}$-*strA*/*aadA7-sul1-tet*(A)-*aac(3)-Ie-gyrA*$_{Ser83\rightarrow Phe83}$	1,600 bp/*aac(3)-Ie-aadA7*	[V-core]-*avrA*
DK	08-00268 (05)	*S.* Newport (U)	Human	LJ/RJ	[Penta] -GEN-KAN/NEO-TMP/SXT-NAL	*bla*$_{PSE-1}$-*floR-strA*/*aadA7-sul1-tet*(A)-*tet*(G)-*aac(3)-Ie-aphA1-dfrA15-gyrA*$_{Asp87\rightarrow Gly87}$	750 bp/*dfrA15* + 1,200 bp/*bla*$_{PSE-1}$ + 1,600 bp/*aac(3)-Ie-aadA7*	[V-core]-*avrA-sodC1-sopE1*
DK	08-00269 (05)	*S.* Newport (U)	Human	LJ/RJ	[Penta] -GEN-KAN/NEO-TMP/SXT-NAL	*bla*$_{PSE-1}$-*floR-strA*/*aadA7-sul1-tet*(A)-*tet*(G)-*aac(3)-Ie-aphA1-dfrA15-gyrA*$_{Asp87\rightarrow Gly87}$	750 bp/*dfrA15* + 1,200 bp/*bla*$_{PSE-1}$ + 1,600 bp/*aac(3)-Ie-aadA7*	[V-core]-*avrA-sodC1-sopE1*
DK	08-00313 (05)	*S.* Newport (U)	Human	LJ/RJ	[Penta] -GEN-TMP/SXT-NAL	*bla*$_{PSE-1}$-*floR-strA*/*aadA7-sul1-tet*(G)-*aac(3)-Ie-dfrA15-gyrA*$_{Ser83\rightarrow Phe83}$	750 bp/*dfrA15* + 1,200 bp/*bla*$_{PSE-1}$ + 1,600 bp/*aac(3)-Ie-aadA7*	[V-core]-*avrA-sodC1-sopE1*
DK	08-00314 (05)	*S.* Newport (U)	Human	LJ/RJ	[Penta] -GEN-KAN/NEO-TMP/SXT-NAL	*bla*$_{PSE-1}$-*floR-strA*/*aadA7-sul1-tet*(A)-*tet*(G)-*aac(3)-Ie-aphA1-dfrA15-gyrA*$_{Asp87\rightarrow Gly87}$	750 bp/*dfrA15* + 1,200 bp/*bla*$_{PSE-1}$ + 1,600 bp/*aac(3)-Ie-aadA7*	[V-core]-*avrA-sodC1-sopE1*
D	08-00325 (04)	*S.* Typhimurium	Sausage	LJ/RJ + R	[Penta]	[Penta-R]-*catA1*	[I]	[V-core]-*avrA-spvC-sodC1*
D	08-00326 (05)	*S.* Typhimurium	Swine	LJ/RJ + R	[Penta] -TMP/SXT	[Penta-R]-*catA1-sul3*-(unknown)[i]	[I]	[V-core]-*avrA-spvC-sodC1*
D	08-00272 (05)	*S.* Typhimurium	Swine	LJ/RJ + R	[Penta] -GEN-TMP/SXT	[Penta-R]-*bla*$_{TEM-1}$-like-*catA1-strA*/*aadA1-sul2-aacC4-dfrA1*	[I] + 1,600 bp/*dfrA1-aadA1*	[V-core]-*avrA-spvC-sodC1*
D	08-02885 (05)	*S.* Typhimurium (U)	Rabbit	LJ/RJ + R	GEN-STR/SPE-SUL	*aadB-strA*/*aadA2-sul1*	800 bp/*aadB* + integron containing *aadA2*[j]	[V-core]-*avrA-spvC-sodC1*
D	08-02886 (05)	*S.* Typhimurium (U)	Rodent	LJ/RJ + R	AMP/AMC-CHL/FLO-SUL-TET-GEN-TMP/SXT	*bla*$_{PSE-1}$-*floR-sul1-tet*(G)-*aadB*-(unknown)	800 bp/*aadB* + 1,200 bp/*bla*$_{PSE-1}$	[V-core]-*avrA-spvC-sodC1*
NL	08-00264 (05)	*S.* Typhimurium	Chicken	LJ/RJ + R	[Penta] -NAL	[Penta-R]-*gyrA*$_{Asp87\rightarrow Tyr87}$	[I]	[V-core]-*avrA-spvC-sodC1*
NL	08-02865 (05)	*S.* Typhimurium (U)	Human	LJ	[Penta] -GEN	[Penta-R]-(unknown)	[I]	[V-core]-*avrA-spvC-sodC1*
NL	08-00315 (05)	*S.* Typhimurium	Rapeseed	LJ/RJ + R	[Penta]	[Penta-R]-*bla*$_{TEM-1}$-like	[I]	[V-core]-*avrA-spvC-sodC1*
NL	08-00312 (05)	*S.* Typhimurium	Human	LJ/RJ + R	[Penta]	[Penta-R]	[I]	[V-core]-*avrA-spvC-sodC1*
NL	08-00316 (04)	*S.* Typhimurium	Human	LJ/RJ + R	[Penta] -(CIP-NAL)[k]	[Penta-R]-*catA1-qnrB19*	[I]	[V-core]-*avrA-spvC-gipA-sodC1*
NL	08-00270 (04)	*S.* Typhimurium	Pig	LJ/RJ + R	[Penta] -TMP/SXT	[Penta-R]-*bla*$_{TEM-1}$-like-*catA1-sul2-tet*(A)-*dfrA14*	[I]	[V-core]-*avrA-spvC-sodC1*
NL	08-00317 (05)	*S.* Typhimurium	Pig	LJ/RJ + R	[Penta]	[Penta-R]-*bla*$_{TEM-1}$-like-*catA1-tet*(A)	[I]	[V-core]-*avrA-spvC-sodC1*
NL	08-00318 (05)	*S.* Typhimurium	Human	LJ/RJ + R	[Penta] -GEN	[Penta-R]-*catA1-strA-tet*(B)-*aacC4*	[I]	[V-core]-*avrA-spvC-sodC1*
NL	08-00319 (05)	*S.* Typhimurium	Pig	LJ/RJ + R	[Penta]	[Penta-R]-*catA1*	[I]	[V-core]-*avrA-spvC-sodC1*
I	08-00322 (04)	*S.* Typhimurium	Human	LJ/RJ + R	[Penta] -TMP/SXT	[Penta-R]-*catA1-sul2-dfrA14*	[I]	[V-core]-*avrA-spvC-sodC1*
I	08-00271 (04)	*S.* Typhimurium	Human	LJ/RJ + R	[Penta] -KAN/NEO-NAL	[Penta-R]-*catA1-strA-sul2-tet*(B)-*aphA1-gyrA*$_{Asp87\rightarrow Asn87}$	[I]	[V-core]-*avrA-spvC-sodC1*

DK	08-00323 (05)	*S.* Typhimurium	Human	LJ/RJ + R	[Penta]	-GEN	[Penta-R]-*catA1-strA-aacC4*	[I]	[V-core]-*avrA-spvC-sodC1*
DK	08-00324 (05)	*S.* Typhimurium	Human	LJ/RJ + R	[Penta]	-GEN	[Penta-R]-*catA1-strA-aacC4*	[I]	[V-core]-*avrA-spvC-sodC1*
UK	08-00320 (04)	*S.* Typhimurium	Sheep	LJ/RJ + R	[Penta]		[Penta-R]-*catA1*	[I]	[V-core]-*avrA-spvC-sodC1*
UK	08-00321 (05)	*S.* Typhimurium	Cattle	LJ/RJ + R	[Penta]		[Penta-R]-*catA1*	[I]	[V-core]-*avrA-spvC-sodC1*
H	08-00327 (06)	*S.* Typhimurium	Goose	LJ/RJ + R	[Penta]		[Penta-R]-bla_{TEM-1}-like	[I]	[V-core]-*avrA-sodC1*
H	08-00328 (06)	*S.* Typhimurium	Goose	LJ/RJ + R	[Penta]		[Penta-R]	[I]	[V-core]-*avrA-sodC1*
H	08-00329 (06)	*S.* Typhimurium	Pork sausage	LJ/RJ + R	[Penta]	-GEN-TMP/SXT	[Penta-R]-*cmlA1-strA-sul3-aacC4-dfrA12*	[I] + integron containing *dfrA12-aadA2-cmlA-aadA1*[l]	[V-core]-*avrA-spvC-sodC1*

[a] For country abbreviations, see note *a* to Table 1.
[b] Markers for the presence of SGI1: S004, SGI1 ORF outside the MDR region; LJ, left junction of SGI1; RJ, right junction of SGI1; R, retron phage element.
[c] *For* abbreviations, see Materials and Methods.
[d] See note *c* toTable 1.
[e] [Penta-R], SGI1 characteristic pentaresistance genotype [bla_{PSE-1}-*floR-aadA2-sul1-tet*(G)].
[f] [I], SGI1 characteristic class 1 integron [1,000 bp/*aadA2* + 1,200 bp/bla_{PSE-1}].
[g] [V-core], virulence genes present throughout all 38 isolates: *ssaQ*, *mgtC*, *spi4_D*, *sopB*, and *bcfC*.
[h] Intermediate (i) resistance phenotype to STR (MIC, 16 μg/ml) (37).
[i] Unknown, none of the genes tested were present.
[j] Defective integron, undetectable by 5′CS/3′CS primers. PCR products were observed using the following primer sets: 5′CS/aadA2-B, int1-R/aadA2-R, and int1-R/qacΔE-R. No PCR product was obtained using aadA2-F/sul1-B and aadA2-F/3′CS primers (see Table SA in the supplemental material).
[k] Decreased susceptibility phenotype to CIP (MIC, 0.5 μg/ml) and NAL (MIC, 8 μg/ml) (12).
[l] Defective integron, undetectable by 5′ CS/3′CS primers, as described by Antunes et al. (5).
[m] (U), unconventional isolate.

RESULTS

Molecular typing. In 33 of the 38 isolates (87%), one to five plasmids were detected, ranging from approximately ≤6 to 180 kb. All *S.* Typhimurium isolates ($n = 23$) carried a serovar-specific virulence plasmid of 94 kb (33, 36). No plasmids were identified in five isolates belonging to the serovars *S*. Newport (3 isolates), *S*. Kentucky (1), and *S*. Derby (1).

PFGE analysis using XbaI generated 26 patterns. The 23 *S.* Typhimurium isolates showed 14 different patterns, the 5 *S*. Derby isolates 4 patterns, the 4 *S*. Newport isolates 3 patterns, and the 3 *S*. Albany isolates 2 different patterns (Fig. 1).

By MLVA analysis, the 23 *S.* Typhimurium isolates were assigned to 21 MLVA types (Table 1). Two of the types (allele numbers 3-13-8-24-311 and 3-11-5-NA-311) were represented by two strains each, isolated in Germany and Hungary. All isolates showed identical alleles for loci STTR-9 and -3. The highest numbers of alleles were found in loci STTR-6 (12 variants) and STTR-10 (13 variants).

Phenotypic and genotypic characterization of antimicrobial resistance. Fourteen different resistance patterns, conferring resistance to at least four antimicrobial substances, were identified among the isolates tested (Tables 1 and 2). The SGI1 characteristic Penta-R phenotype was detected in 29 isolates throughout all serotypes. Variations within this phenotype were observed in several isolates (Tables 1 and 2).

The prevalence of resistance genes and their distributions in the different *Salmonella* serovars are shown in Table 2. Twenty-five isolates were positive for the five characteristic SGI1 resistance genes, bla_{PSE-1}, *floR*, *aadA2*, *sul1*, and *tet*(G), but again, variations were found (Table 2). The *sul1* gene was present in all 38 isolates. The plasmid-mediated-quinolone-resistance (PMQR) gene, *qnrB19*, was detected on a 2.7-kb ColE-like plasmid in a Dutch *S.* Typhimurium isolate (CIP MIC = 0.5 μg/ml; NAL MIC = 8 μg/ml) of human origin (25).

All isolates tested in this study harbored class 1 integrons (Table 2), whereas none carried class 2 integrons. Based on the gene cassette content of the variable region and the amplicon size, the nine different types of class 1 integrons shown in Table 3 were observed. The two class 1 integrons typically linked to the presence of SGI1, generating PCR amplicons of 1,200 bp and 1,000 bp and carrying the bla_{PSE-1} and *aadA2* gene cassettes, respectively, were present in 25 (66%) of the isolates. Two *S.* Typhimurium isolates carried 3′-CS-defective integrons that could not be detected using the 5′-CS/3′-CS primers but were identified by linking the integrase gene *intI1* and the different gene cassettes. One isolate carried the *aadA2* and bla_{PSE-1} variable regions characteristic of the SGI1 integron, together with an additional defective integron with the *dfrA12-aadA2-cmlA1-aadA1* variable region (5), while the *aadB* variable region and a defective integron containing *aadA2* were present in the other (Tables 2 and 3).

Due to the differences in the SGI1 MDR gene content, the *S.* Albany (three isolates), *S*. Kentucky (1), *S*. Newport (4), and *S.* Typhimurium (2) isolates were considered part of the unconventional isolates (Table 2).

Analyses of SGI1 flanking regions on unconventional isolates lacking a classical chromosomal attachment site. In the other five unconventional isolates, one *S*. Agona (08-02864), three *S*. Derby (08-02866, 08-02867, and 08-02868), and one *S.*

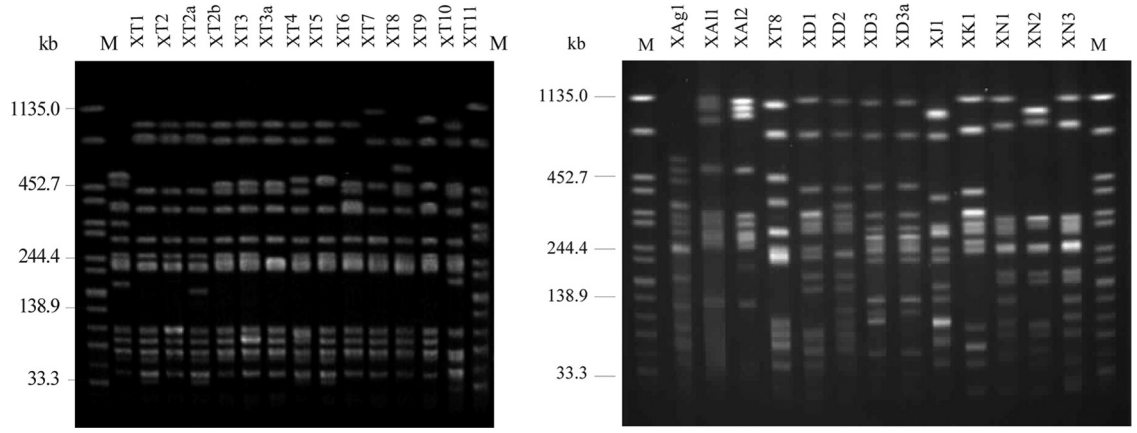


FIG. 1. XbaI PFGE profiles (X) of representative isolates. To define the profiles, only bands at >33 kb were considered. Similar profiles with only a one-band difference are designated by letters. Profiles with differences in two or more bands are designated by numbers. Lanes M contained XbaI-digested DNA of *S. enterica* serovar Braenderup H9812, which was used as a size standard. T, *S.* Typhimurium; Ag, *S.* Agona; Al, *S.* Albany; D, *S.* Derby; J, *S.* Paratyphi B dT+ (*S.* Java); K, *S.* Kentucky; N, *S.* Newport.

Typhimurium (08-02865), one or both junctions were absent. The flanking regions of these isolates were analyzed by PCR screening. In all five isolates, the normal SGI1 attachment sites *thdF* and *yidY*, as well as all analyzed up- and downstream flanking genes (*dnaN*, *dnaA*, *rpmH*, *rnpA*, *yidC*, *thdF*, *int2*, *urt*, *rt*, *yidY*, *yidZ*, *yieE*, *yieF*, *yieG*, and *yieH*), were present.

Integration of SGI1 into a secondary chromosomal attachment site between the genes *sodB* and *purR* was not observed, because in all isolates, a PCR product of 518 bp was generated.

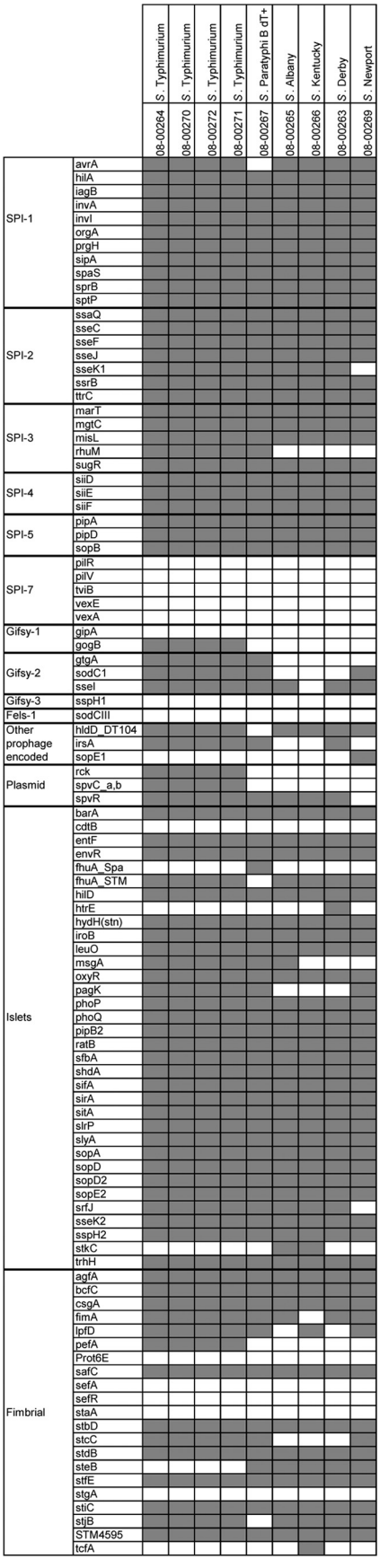
Virulotyping. All isolates were screened by PCR analysis for the presence or absence of 10 selected virulence genes. The *ssaQ*, *mgtC*, *spi4_D*, *sopB* (carried by *Salmonella* pathogenicity islands [SPIs]), and *bcfC* (fimbria-related) genes were present in all the isolates tested. Except for the *S*. Paratyphi B dT+ strain, all isolates also carried the *avrA* gene located in SPI1. The *spvC* gene, carried by the *Salmonella* virulence plasmid, was present in all *S.* Typhimurium isolates except two. Likewise, the *sodC1* gene, located on a bacteriophage, was found in all isolates belonging to *S.* Typhimurium, *S*. Newport, and *S*. Paratyphi B dT+, whereas for *sopE1*, only the four *S.* Newport and three *S*. Derby isolates tested positive. The *gipA* gene was detected in only one *S.* Typhimurium isolate. Altogether, in this strain collection, seven different combinations of virulence genes were detected (Table 2).

Since major serovar-specific differences were observed, nine representative *Salmonella* isolates were analyzed by DNA microarray in more detail. All four *S.* Typhimurium isolates tested had identical virulence gene repertoires (Fig. 2). In contrast to the five isolates of other serovars tested, all *S*. Typhimurium isolates possessed *gogB* (encoding a leucine-rich-repeat protein), usually located on Gifsy-1, but lacked *gipA* (encoding a Peyer's patch-specific virulence factor, GipA). Virulence markers associated with the prophage Gifsy-2 were always present in *S.* Typhimurium, but no markers of the Gifsy-3 and Fels-1 prophages tested positive. Other prophage genes, like *hldD*_DT104 (encoding a DT104-specific phage protein) and *irsA* (encoding a putative transcriptional regulator and internal response element to stress) were present, but *sopE1* (encoding a translocated effector protein) was absent. The *Salmonella* virulence plasmid genes *rck*, *spvC*, and *spvR* were positive, and with the exception of *cdtB* (encoding a cytolethal distending toxin), *fhuA*_Spa (encoding an outer membrane protein receptor), *htrE* (encoding a probable porin/fimbrial assembly protein), and *stkC*

TABLE 3. Class 1 integrons detected in SGI1-positive isolates (n = 38)

Variable-region size(s)[a] (bp)	Gene cassette(s)[a]	n	Serovar(s)
1,000 + 1,200	*aadA2* + bla_{PSE-1}	23	*S.* Typhimurium (19), *S.* Derby (2), *S.* Agona, *S.* Paratyphi B dT+
1,000 + 1,200 + 1,600	*aadA2* + bla_{PSE-1} + *dfrA1-aadA1*	1	*S.* Typhimurium
1,000 + 1,200 + defective integron[b]	*aadA2* + bla_{PSE-1} + *dfrA12-aadA2-cmlA-aadA1*[b]	1	*S.* Typhimurium
1,200 + 1,250	bla_{PSE-1} + *dfrA1-orf*	3	*S.* Albany
1,000	*aadA2*	3	*S.* Derby
1,600	*aac(3)-Ie-aadA7*	1	*S.* Kentucky
1,600 + 1,200 + 750	*aac(3)-Ie-aadA7* + bla_{PSE-1} + *dfrA15*	4	*S.* Newport
1,200 + 800	bla_{PSE-1} + *aadB*	1	*S.* Typhimurium
800 + defective integron[c]	*aadB* + *aadA2*[c]	1	*S.* Typhimurium

[a] PCR/sequencing was performed using the 5′CS/3′CS primers (30).
[b] Defective integron, undetectable by 5′CS/3′CS primers, as described by Antunes et al. (5).
[c] Defective integron, undetectable by 5′CS/3′CS primers. PCR products were observed using the following primer sets: 5′CS/aadA2-B, int1-R/aadA2-R, and int1-R/qacΔE-R.



(encoding an outer membrane usher protein), all genes analyzed on virulence islets were present. For the fimbria-coding genes tested, *pefA* (encoding a virulence plasmid fimbrial protein) was positive and *steB* (encoding an outer membrane fimbrial usher protein) was negative, in contrast to the isolates of other serovars.

The *S.* Paratyphi B dT+ isolate was solely negative for *avrA* (encoding a protein that inhibits activation of NF-κB), *hldD*_DT104, and the fimbrial *stjB* (encoding a putative fimbrial usher protein), and it had none of the Gifsy-1 genes tested. For the islet genes, the only difference from *S.* Typhimurium was *fhuA*_Spa instead of *fhuA*_STM.

S. Albany was characterized by a truncated SPI-3 missing *rhuM* (encoding a putative cytoplasmic protein) and possessed only *sseI* of Gifsy-2 and *hldD*_DT104 of the other prophage genes. In contrast to *S.* Typhimurium and *S.* Paratyphi B dT+, no *pagK* (encoding a PhoPQ-activated protein) was detected, but *stkC* was present. Also, *lpfD* (encoding a long polar fimbrial operon protein), *pefA*, and *stcC* (encoding a putative outer membrane protein) of the fimbrial genes were missing.

For *S.* Kentucky, five genes were found to be different from those of *S.* Albany, namely, the islet gene *msgA* (encoding an *ssrB*-regulated factor) and *fimA* (encoding a major type 1 subunit fimbrin) were negative, while *lpfD* and *tcfA* (encoding an *S.* Typhi colonization factor and putative fimbrial protein) were positive. The Gifsy-2 gene *sseI* was negative only for this isolate.

The *S.* Derby strain analyzed had the same fimbrial set as *S.* Albany. In addition, the isolate was positive for *irsA* and solely for *htrE*. The genes *msgA* and *pagK* were negative.

Also, one *S.* Newport strain was investigated for its virulence genes. Probes were negative for *sseK1* (encoding *Salmonella*-secreted effector K1) of SPI-2 and *rhuM* of SPI-3. Of the Gifsy-2 genes, *gtgA* was missing, but genes of other prophages were positive in the case of *hldD*_DT104 and, only in this isolate, *sopE1*. Additionally, it possessed no *srfJ* gene (encoding a putative virulence factor activated by transcription factor SsrB), which was found only for this isolate.

DISCUSSION

The acquisition of genomic islands has played a central role in bacterial evolution as a mechanism of diversification and adaptation (10). Since the emergence of pandemic multidrug-resistant *S.* Typhimurium DT104, its antimicrobial resistance-conferring genomic island, SGI1, has increasingly attracted research interest. In particular, the horizontal-transfer potential of SGI1 has been intensively studied (8, 16). In this study, we sought to investigate the molecular properties of SGI1-carrying epidemic *Salmonella* isolates in a European strain collection of human, animal, and food origin. We specially focused on isolates that belonged to serovars other than *S.* Typhimurium or to phage types of *S.* Typhimurium other than

FIG. 2. Virulence determinant microarray data for 9 SGI1-positive *Salmonella* strains. The analyzed genes are grouped according to their genomic locations (SPI-1 to SPI-7; prophages Gifsy-1, Gifsy-2, Gifsy-3, and Fels-1; plasmids; and islets) or function (fimbrial).

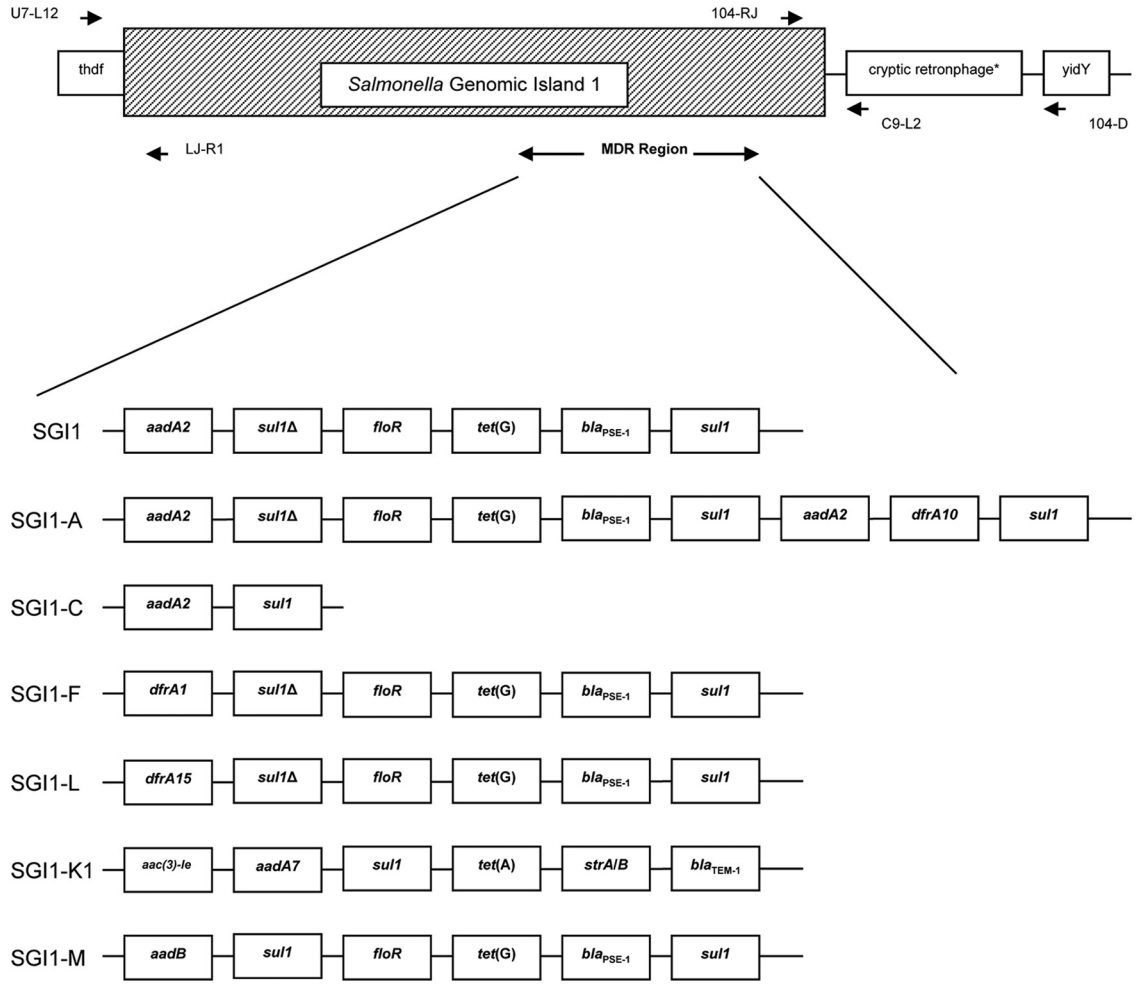


FIG. 3. SGI1 variants corresponding to the resistance gene repertoire found within the isolates (*S.* Agona and *S.* Paratyphi B dT+, SGI1; *S.* Typhimurium, SGI1 and SGI1-M; *S.* Derby, SGI1-A and SGI1-C; *S.* Albany, SGI1-F; *S.* Newport, SGI1-L; *S.* Kentucky, SGI1-K1). The scheme is based on information from Amar et al. (3), Boyd et al. (9), Cloeckaert et al. (13), Doublet et al. (18), and Vo et al. (39). Only antimicrobial resistance genes are shown. ∗, not present in all isolates.

DT104. We intended to establish the relationship between virulence, multidrug resistance, and the presence of SGI1.

The results of typing showed wide variability within the series (38 isolates from seven serovars). PFGE analysis distinguished within the same serovar several XbaI patterns, with a total of 26 different patterns. MLVA analysis of the *S.* Typhimurium isolates was also very heterogeneous (21 types). These findings are in line with the supposed epidemiological nonrelatedness of the strains, which were isolated in different countries and from different sources. In contrast, characterization of the phenotypic and genotypic antimicrobial resistance in general displayed certain serovar-specific features. Apart from the resistance genes organized in SGI1, several strains exhibited additional resistance genes with either chromosomal or plasmidic locations. Altogether, we could identify eight possible SGI1 variants in our strain collection, based on the particular resistance genotype and the presence of class 1 integrons. The majority of strains (61%; 23 isolates), belonging to the *S. enterica* serovars Agona (1 isolate), Paratyphi B dT+ (1), and Typhimurium (21), harbored the classical SGI1 resistance genes (Fig. 3) (3, 34). Isolates with a gene repertoire typical of the variants SGI1-A and SGI1-C (Fig. 3) were found only within *S.* Derby (one each). The three *S.* Albany strains harbored genes suggesting the presence of SGI1-F (Fig. 3) (14). SGI1-L was recognized in the four *S.* Newport isolates and SGI1-K1 in *S.* Kentucky (Fig. 3), as previously described (13, 18). In the *S.* Typhimurium isolate 08-02886, the resistance gene cluster found (Fig. 3) suggests the presence of the variant SGI1-M, which was first described for a Dutch *S.* Typhimurium DT104 horse isolate by Vo et al. (39). To our knowledge, this is the first time that an SGI1-M variant has been detected in a German *S.* Typhimurium DT120 isolate of rodent origin. The same variant was also detected by Rodríguez et al. (35) in German *S.* Typhimurium isolates (phage types DT104L and DT012) of equine, cat, and avian origin associated with the presence of the extended-spectrum beta-lactamase-encoding gene $bla_{\text{CTX-M-1}}$. An integron with a variable region of 800 bp containing the *aadB* gene cassette was also found in another *S.* Typhimurium isolate, 08-02885, which additionally carried a 3′-CS-defective integron with an *aadA2* gene cassette. We as-

sume that this isolate contains a novel variant similar to SGI1-C (34) with additional acquired resistance to gentamicin, but this assumption needs further investigation. The SGI1 has historically been linked to *S.* Typhimurium, and we have demonstrated that, despite the clonal diversity of the *S.* Typhimurium isolates (shown by PFGE and MLVA analyses), there is low genetic variation in the genomic island, since among 23 isolates, 21 carry the classical SGI1, 1 the SGI1-M variant, and another, as mentioned above, a potential new variant.

The virulotyping results for strains carrying SGI1 indicated that only little or no variation was found for most genes incorporated in SPIs and for the fimbrial marker. Thus, the genes *ssaQ* , *mgtC* , *spi4_D* , *sopB* , and *bcfC* were present throughout all serovars in all 38 isolates. It was observed that some virulence patterns were serovar specific, e.g., all *S.* Newport and most *S.* Typhimurium strains shared a common virulotype, which was characterized in both by the presence of eight genes. The association of a certain serovar with a virulence-associated gene panel was also confirmed by the microarray results in this study and a previous study conducted by Huehn et al. (27) within the Med-Vet-Net project on a wide collection (77 isolates, 10 of them SGI positive) of European *Salmonella* isolates belonging to different serovars. The presence of genes in the SGI1 that can play a role in the virulence of the carrier isolates has been mentioned previously (6, 20). The data presented here and by Huehn et al. (27) show that, despite minor differences, SGI1-positive isolates carry more or less identical virulence gene repertoires. Mapping of the unconventional isolates did not reveal any aberrations either in the SGI1 integration sites (8) or in the flanking regions. Nevertheless, in some isolates, the left and right junctions (1 *S* . Agona and 3 *S* . Derby isolates) or only the right junction (one *S.* Typhimurium isolate) could not be detected by conventional PCR, although the presence of SGI1 was proved by real-time PCR targeting S004 in the precursor study carried out by Amar et al. (3). For the *S.* Typhimurium isolate in which only the left junction was detectable, one might assume genetic rearrangements or single-nucleotide polymorphisms (SNPs) at the ends of SGI1. For the *S* . Derby and the *S* . Agona isolates, we proved SGI1 was not integrated in a secondary chromosomal attachment site between the genes *sodB* and *purR* , as suggested by Doublet et al. (17). To elucidate the exact genetic organization of this region, further mapping and sequencing experiments will be undertaken.

In summary, we set up a panel of strains from different origins that can be used as a reference collection for molecular studies. We could observe great phenotypic and genetic heterogeneity of SGI1-positive isolates. Apart from the resistance genes typical of SGI1 [bla_{PSE-1}, *floR*, *sul1*, *tet*(G), and *aadA2*], other chromosomal or plasmid-located resistance determinants were detected. As shown by other authors (9, 10, 13–18, 34, 39), several genetic events led to a large number of SGI1 variants, which can be spread through vertical and horizontal transfer. Due to their virulence gene repertoires and their resistance properties, some of these variants/isolates may become clinically relevant. The data presented provide a solid basis for cooperation in resistance and virulence studies leading to a scientifically based risk characterization.

ACKNOWLEDGMENTS

We thank A. Gramatke, K. Thomas, W. Barownick, E. Junker, M. Jaber, J. Ledwolorz, and G. Berendonk (*Salmonella* and Antimicrobial Resistance Reference Laboratories of the Federal Institute for Risk Assessment) for their assistance. We also thank the Med-Vet-Net WP21 Project Group, which provided the isolates used in this study, and especially F. Aarestrup (DTU, Lyngby, Denmark), M. Kirchner (VLA, Weybridge, United Kingdom), S. Granier (AFSSA, Maison-Alfort, France), I. Luzzi (ISS, Rome, Italy), M. Moreno (UCM, Madrid, Spain), B. Nagy (VMRI, Budapest, Hungary), M. Torpdahl (SSI, Copenhagen, Denmark), J. Szych (PZH, Warsaw, Poland), and K. Hopkins and C. Amar (HPA, London, United Kingdom). We also thank A. Cloeckaert, R. La Ragione, and E. Liebana for their invaluable advice.

This work was funded by the Federal Institute for Risk Assessment (BfR: 46-001 and 45-004) and the EU Network of Excellence Med-Vet-Net (WP21 and -26).

REFERENCES

1. **Ahmed, A. M., A. I. Hussein, and T. Shimamoto.** 2007. *Proteus mirabilis* clinical isolate harbouring a new variant of *Salmonella* genomic island 1 containing the multiple antibiotic resistance region. J. Antimicrob. Chemother. **59:**184–190.
2. **Akiba, M., et al.** 2006. Detection and characterization of variant *Salmonella* genomic island 1s from *Salmonella* Derby isolates. Jpn. J. Infect. Dis. **59:** 341–345.
3. **Amar, C. F., et al.** 2008. 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*. Microb. Drug Resist. **14:**79–92.
4. **Anderson, E. S., L. R. Ward, M. J. de Saxe, and J. D. de Sa.** 1977. Bacteriophage-typing designations of *Salmonella typhimurium*. J. Hyg. **78:**297–300.
5. **Antunes, P., J. Machado, and L. Peixe.** 2007. Dissemination of *sul3*-containing elements linked to class 1 integrons with an unusual 3′ conserved sequence region among *Salmonella* isolates. Antimicrob. Agents Chemother. **51:**1545–1548.
6. **Bamforth, J., et al.** 2009. *Salmonella* Genomic Island 1 influences expression of virulence-associated genes in early stationary phase and enhances killing of *Caenorhabditis elegans* for *Salmonella enterica* serovar Typhimurium DT104, p. 104. *In* Proceedings of the 3rd ASM Conference on *Salmonella:* Biology, Pathogenesis, and Prevention. ASM Press, Washington, DC.
7. **Boyd, D. A., G. A. Peters, L. Ng, and M. R. Mulvey.** 2000. Partial characterization of a genomic island associated with the multidrug resistance region of *Salmonella enterica* Typhimurium DT104. FEMS Microbiol. Lett. **189:**285–291.
8. **Boyd, D., et al.** 2001. Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona. J. Bacteriol. **183:**5725–5732.
9. **Boyd, D., A. Cloeckaert, E. Chaslus-Dancla, and M. R. Mulvey.** 2002. Characterization of variant *Salmonella* genomic island 1 multidrug resistance regions from serovars Typhimurium DT104 and Agona. Antimicrob. Agents Chemother. **46:**1714–1722.
10. **Boyd, E. F., S. Almagro-Moreno, and M. A. Parent.** 2009. Genomic islands are dynamic, ancient integrative elements in bacterial evolution. Trends Microbiol. **17:**47–53.
11. **Clinical and Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 7 th ed. (M7-A7), vol. 26, no. 2. CLSI, Wayne, PA.
12. **Clinical and Laboratory Standards Institute.** 2006. Performance standards for antimicrobial susceptibility testing, 16th international supplement (M100-S16), vol. 26, no. 3. CLSI, Wayne, PA.
13. **Cloeckaert, A., K. Praud, B. Doublet, M. Demartin, and F. W. Weill.** 2006. Variant *Salmonella* genomic island 1-L antibiotic resistance gene cluster in *Salmonella enterica* serovar Newport. Antimicrob. Agents Chemother. **50:** 3944–3946.
14. **Doublet, B., et al.** 2003. Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster in *Salmonella enterica* serovar Albany. Emerg. Infect. Dis. **9:**585–591.
15. **Doublet, B., et al.** 2004. *Salmonella* genomic island 1 multidrug resistance gene clusters in *Salmonella enterica* serovar Agona isolated in Belgium in 1992 to 2002. Antimicrob. Agents Chemother. **48:**2510–2517.
16. **Doublet, B., D. Boyd, M. R. Mulvey, and A. Cloeckaert.** 2005. The *Salmonella* genomic island 1 is an integrative mobilizable element. Mol. Microbiol. **55:**1911–1924.
17. **Doublet, B., G. R. Golding, M. R. Mulvey, and A. Cloeckaert.** 2008. Secondary chromosomal attachment site and tandem integration of the mobilizable *Salmonella* genomic island 1. PLoS One **3:**e2060.
18. **Doublet, B., et al.** 2008. Novel insertion sequence- and transposon-mediated

genetic rearrangements in genomic island SGI1 of *Salmonella enterica* serovar Kentucky. Antimicrob. Agents Chemother. **52:**3745–3754.
19. **Emborg, H.-D., and A. M. Hammerum (ed.).** 2007. DANMAP 2006. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. National Food Institute, Technical University of Denmark, Søborg, Denmark. http://www.danmap.org/pdfFiles/Danmap_2006.pdf.
20. **Giraud, E., et al.** 2009. *Salmonella* Genomic Island 1 modulates the expression of virulence-related loci in acidic conditions in *Salmonella enterica* serovar Typhimurium DT104, p. 116. *In* Proceedings of the 3rd ASM Conference on *Salmonella:* Biology, Pathogenesis, and Prevention. ASM Press, Washington, DC.
21. **Glynn, M. K., et al.** 1998. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. N. Engl. J. Med. **338:**1333–1338.
22. **Grimont, P. A. D., and F.-X. Weill.** 2007. Antigenic formulae of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur, Paris, France.
23. **Guerra, B., B. Malorny, A. Schroeter, and R. Helmuth.** 2003. Multiple resistance mechanisms in fluoroquinolone-resistant *Salmonella* isolates from Germany. Antimicrob. Agents Chemother. **47:**2059.
24. **Guerra, B., E. Junker, A. Miko, R. Helmuth, and M. C. Mendoza.** 2004. Characterization and localization of drug resistance determinants in multidrug-resistant, integron-carrying *Salmonella enterica* serotype Typhimurium strains. Microb. Drug Resist. **10:**83–91.
25. **Hammerl, J. A., et al.** 2010. pSGI15, a small ColE-like *qnrB19* plasmid of a *Salmonella enterica* serovar Typhimurium strain carrying *Salmonella* genomic island 1 (SGI1). J. Antimicrob. Chemother. **65:**173–175.
26. **Huehn, S., C. Bunge, E. Junker, R. Helmuth, and B. Malorny.** 2009. Poultry associated *Salmonella enterica* subsp. *enterica* serovar 4,12:d:− reveals high clonality and a distinct pathogenicity gene repertoire. Appl. Environ. Microbiol. **75:**1011–1020.
27. **Huehn, S., et al.** 2010. Virulotyping and antimicrobial resistance typing of *Salmonella enterica* serovars relevant to human health in Europe. Foodborne Pathog. Dis. **7:**523–535.
28. **Kado, C. I., and S. T. Liu.** 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. **145:**1365–1373.
29. **Larsson, J. T., et al.** 2009. Development of a new nomenclature for *Salmonella* Typhimurium multilocus variable number of tandem repeats analysis (MLVA). Euro Surveill. **14:**19174.
30. **Levesque, C., L. Piche, C. Larose, and P. H. Roy.** 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. **39:**185–191.
31. **Levings, R. S., D. Lightfoot, S. R. Partridge, R. M. Hall, and S. P. Djordjevic.** 2005. The genomic island SGI1, containing the multiple antibiotic resistance region of *Salmonella enterica* serovar Typhimurium DT104 or variants of it, is widely distributed in other *S. enterica* serovars. J. Bacteriol. **187:**4401–4409.
32. **Lindstedt, B. A., T. Vardund, L. Aas, and G. Kapperud.** 2004. Multiple-locus variable-number tandem-repeats analysis of *Salmonella enterica* subsp. *enterica* serovar Typhimurium using PCR multiplexing and multicolor capillary electrophoresis. J. Microbiol. Methods **59:**163–172.
33. **McClelland, M., et al.** 2001. Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. Nature **413:**852–856.
34. **Mulvey, M. R., D. A. Boyd, A. B. Olson, B. Doublet, and A. Cloeckaert.** 2006. The genetics of *Salmonella* genomic island 1. Microbes Infect. **8:**1915–1922.
35. **Rodríguez, I., et al.** 2009. Extended-spectrum β-lactamases and AmpC β-lactamases in ceftiofur-resistant *Salmonella enterica* isolates from food and livestock obtained in Germany during 2003-07. J. Antimicrob. Chemother. **64:**301–309.
36. **Rotger, R., and J. Casadesús.** 1999. The virulence plasmids of *Salmonella*. Int. Microbiol. **2:**177–184.
37. **Schroeter, A., B. Hoog, and R. Helmuth.** 2004. Resistance of *Salmonella* isolates in Germany. J. Vet. Med. B Infect. Dis. Vet. Public Health **51:**389–392.
38. **Threlfall, E. J.** 2002. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. FEMS Microbiol. Rev. **26:**141–148.
39. **Vo, A. T., E. van Duijkeren, A. C. Fluit, and W. Gaastra.** 2007. A novel *Salmonella* genomic island 1 and rare integron types in *Salmonella* Typhimurium isolates from horses in The Netherlands. Antimicrob. Agents Chemother. **59:**594–599.
40. **Weill, F. X., L. Fabre, B. Grandry, P. A. Grimont, and I. Casin.** 2005. Multiple-antibiotic resistance in *Salmonella enterica* serotype Paratyphi B isolates collected in France between 2000 and 2003 is due mainly to strains harboring *Salmonella* genomic islands 1, 1-B, and 1-C. Antimicrob. Agents Chemother. **49:**2793–2801.
41. **White, P. A., C. J. McIver, and W. D. Rawlinson.** 2001. Integrons and gene cassettes in the *Enterobacteriaceae*. Antimicrob. Agents Chemother. **45:**2658–2661.