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A Predominant Multidrug-Resistant *Salmonella enterica* Serovar Saintpaul Clonal Line in German Turkey and Related Food Products[▽]

Janine Beutlich, Irene Rodríguez, Andreas Schroeter, Annemarie Käsbohrer, Reiner Helmuth, and Beatriz Guerra*

Federal Institute for Risk Assessment (BfR), Diedersdorfer Weg 1, D-12277 Berlin, Germany

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Recently, *Salmonella enterica* subsp. *enterica* serovar Saintpaul has increasingly been observed in several countries, including Germany. However, the pathogenic potential and epidemiology of this serovar are not very well known. This study describes biological attributes of *S.* Saintpaul isolates obtained from turkeys in Germany based on characterization of their pheno- and genotypic properties. Fifty-five *S.* Saintpaul isolates from German turkeys and turkey-derived food products isolated from 2000 to 2007 were analyzed by using antimicrobial agent, organic solvent, and disinfectant susceptibility tests, isoelectric focusing, detection of resistance determinants, plasmid profiling, pulsed-field gel electrophoresis (PFGE), and hybridization experiments. These isolates were compared to an outgroup consisting of 24 *S.* Saintpaul isolates obtained from humans and chickens in Germany and from poultry and poultry products (including turkeys) in Netherlands. A common core resistance pattern was detected for 27 German turkey and turkey product isolates. This pattern included resistance (full or intermediate) to ampicillin, amoxicillin-clavulanic acid, gentamicin, kanamycin, nalidixic acid, streptomycin, spectinomycin, and sulfamethoxazole and intermediate resistance or decreased susceptibility to ciprofloxacin (MIC, 2 or 1 μg/ml, respectively) and several third-generation cephalosporins (including ceftiofur and cefoxitin [MIC, 4 to 2 and 16 to 2 μg/ml, respectively]). These isolates had the same core resistance genotype, with bla_{TEM-1}, *aadB*, *aadA2*, *sul1*, a Ser83→Glu83 mutation in the *gyrA* gene, and a chromosomal class 1 integron carrying the *aadB-aadA2* gene cassette. Their XbaI, BlnI, and combined XbaI-BlnI PFGE patterns revealed levels of genetic similarity of 93, 75, and 90%, respectively. This study revealed that a multiresistant *S.* Saintpaul clonal line is widespread in turkeys and turkey products in Germany and was also detected among German human fecal and Dutch poultry isolates.

Over the last few decades, the emergence and spread of antimicrobial agent-resistant zoonotic bacteria has become a serious public health concern (2, 23). The widespread use of antimicrobial agents for disease control, including at the farm level, has increased selection of antimicrobial agent-resistant *Salmonella* isolates (1, 23, 44). Food animals are considered an important reservoir for resistant bacteria. These animals and food products derived from them are traded worldwide, which contributes to the global spread of zoonotic agents and antimicrobial resistance. In the last few years, several monitoring activities were initiated in order to generate baseline data on antimicrobial resistance in bacteria isolated from livestock and food derived from animals that could be used in future assessments of the risk of antimicrobial resistance (10).

According to European Union (EU) Zoonoses Regulation (EC) no. 2160/2003 on the control of *Salmonella* and other specified food-borne zoonotic agents, a European Community target for reducing the prevalence of *Salmonella* in turkey flocks had to be established. Consequently, EU Commission decision 2006/662/EC was released, and a baseline survey of the prevalence of *Salmonella* in turkey flocks was carried out in all European countries, including Germany, over a 1-year period starting on 1 October 2006 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178706574172.htm). The main objective of this study was to estimate the prevalence of *Salmonella* in commercial flocks of turkeys. The data showed that at the EU level *Salmonella enterica* serovar Bredeney was the serovar reported most frequently for fattening turkey flocks and occurred in 17.2% of the samples from *Salmonella*-positive flocks (1,084 of 3,702 flocks were positive), followed by *S. enterica* serovar Hadar, *S. enterica* serovar Derby, and then *S. enterica* serovar Saintpaul (14.0%, 11.3%, and 10.4% of the samples from positive flocks, respectively). In this study, *S.* Saintpaul was detected in fattening turkeys in 12 countries, reflecting the wide spread of this serovar. Recently, *S.* Saintpaul has been increasingly observed in several countries, including Germany. According to Enter-Net reports (data on *Salmonella* human isolates identified by European national reference centers), for the last quarter of the year 2006 *S.* Saintpaul was the fourth most common serovar (1.6%) and, in contrast to the data for previous years, was one of the most frequent causes of human salmonellosis in Europe. After this, its prevalence was 1.2% and 0.6% in the first quarters of 2007 and 2008, respectively, among the *Salmonella* serotypes implicated in human disease (http://ecdc.europa.eu/en/publications/Pages/Surveillance_Reports.aspx). During the period from 2001 to 2009 in Germany, 463 cases of human salmonellosis related to *S.* Saintpaul (0.09% of all cases; the maximum prevalence was 0.15% in 2008, the prevalence was 0.1% in 2002, 2005, 2006, and 2009 and 0.06% in 2004, and the minimum prevalence was 0.05% in 2007) were reported to the Robert Koch Institute (Berlin, Germany) (www3.rki.de/SurvStat). In Germany, *S.* Saint-

* Corresponding author. Mailing address: Federal Institute for Risk Assessment (BfR), Diedersdorfer Weg 1, D-12277 Berlin, Germany. Phone: 49-30-8412-2082. Fax: 49-30-8412-2953. E-mail: beatriz.guerra@bfr.bund.de.

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paul attracted public attention particularly in 1993, when it caused a nationwide food-borne outbreak (27). This serotype has often been related to outbreaks in other countries, and in 2008 it was implicated in a large multistate human outbreak associated with various vegetables in the United States (4).

Previous studies showed that isolates of *S.* Saintpaul are often multidrug resistant (33, 35), but little is known about the mechanisms underlying antimicrobial resistance or about the pathogenic potential and epidemiology of isolates belonging to this serotype. The goals of this study were to obtain information about the resistance characteristics of isolates collected between 2000 and 2007 in Germany and to assess possible clonal relationships. The isolates used originated from turkey feces collected during the German *Salmonella* baseline study (in 2006 and 2007) or from diagnostic samples, including samples of turkey feces and turkey-related food products. These isolates were compared with German strains isolated from humans and chickens and with poultry strains isolated in Netherlands.

MATERIALS AND METHODS

Bacterial isolates and selection of isolates. Fifty-five *S.* Saintpaul isolates collected in Germany from 2000 to 2007 were analyzed. All of the isolates originated from turkey (33 feces isolates) or turkey meat (22 food isolates). They were selected from isolates submitted to the *Salmonella* Reference Laboratory (NRL-Salm of the Federal Institute for Risk Assessment) for typing. Duplicate isolates that were putative siblings (isolates obtained from the same place on the same date and exhibiting the same resistance profile) were not included in the study. All isolates included had routinely been tested previously for susceptibility to 17 antimicrobial agents by the broth microdilution method by following the Clinical and Laboratory Standards Institute (CLSI) guidelines (13) as described by Schroeter et al. (37). The antimicrobials tested and MIC breakpoints are shown in Table 1.

The strains included in this study were randomly selected and represented the different resistance phenotypes found in each of the three defined sampling groups (Tables 1 and 2). Group 1 consisted of 16 isolates obtained from fecal samples from the 2006-2007 EU turkey baseline study (11 *S*. Saintpaul isolates and 5 rough isolates confirmed to be *S*. Saintpaul isolates by molecular methods, which represented [without siblings] the 19 *S*. Saintpaul isolates and 12 *S*. rough isolates collected in the baseline study). Group 2 comprised 17 turkey feces isolates randomly selected from routine submissions (11 of 167 *S*. Saintpaul isolates from turkey feces submitted in 2002 and 2003 and 6 of 46 isolates from turkey feces submitted in 2005 and 2006). Group 3 included 22 turkey-derived food isolates randomly selected from routine submissions of 147 *S*. Saintpaul isolates from turkey food products from 2000 to 2007.

Additionally, eight *S*. Saintpaul isolates from human stool samples (isolated in 2006 and 2007 and provided by the Robert Koch Institute, Wernigerode, Germany) and two *S*. Saintpaul isolates from chickens (2006), all isolated in Germany, as well as 14 Dutch *S*. Saintpaul isolates (provided by the Central Veterinary Institute of Wageningen, Lelystad, Netherlands) that originated from poultry and poultry products (in 2006 to 2008), were added as outgroup strains for comparison.

Antimicrobial susceptibility testing. All isolates were tested by the disk diffusion method (15) to determine their susceptibilities to a panel of 14 β-lactams (Oxoid Ltd., London, England). The antimicrobial agents, concentrations, and breakpoints used (16) are shown in Table 3. A phenotypic test to confirm the presence of extended-spectrum beta-lactamases (ESBLs) was performed using the double-disk technique by placing amoxicillin-clavulanic acid disks (30 μg) 30 mm from ceftazidime (30 μg) and cefotaxime (30 μg) disks. An increase in the size of the cephalosporin zone close to the disk containing the β-lactamase inhibitor (synergy) indicates the presence of ESBLs (www.medvetnet.org/pdf/Reports/Appendix_2_Workpackage_9.doc).

Further characterization of the MIC values of six selected β-lactams (Sigma-Aldrich, Taufkirchen, Germany) was performed only for the 55 *S.* Saintpaul isolates from German turkey or turkey food products by using the agar dilution method (13). The antimicrobials tested (Table 3) included first-generation (cephalothin), second-generation (cefuroxime), and third-generation (cefoxitin, cefotaxime, ceftiofur, and ceftazidime) cephalosporins. The antimicrobial concentrations and MIC breakpoints used (16) are shown in Table 3. *Escherichia coli* ATCC 25922 was used as the control strain.

Susceptibility to organic solvents and triclosan. All *S*. Saintpaul isolates were tested to determine their susceptibilities to organic solvents and the disinfectant triclosan. The susceptibility to *n*-hexane and cyclohexane was analyzed as previously described (8, 29). *E. coli* AG100 (wild-type *E. coli* K-12) and AG102 (*marR1* mutant of AG100) were used as controls (42). Triclosan (Irgasan; Sigma-Aldrich, Taufkirchen, Germany) susceptibility tests were carried out by using the CLSI agar dilution method (13). The concentrations tested in Mueller-Hinton agar ranged from 0.015 to 64 μg/ml. Isolates were considered to have a high level of resistance (MIC, >32 μg/ml), a medium level of resistance (MIC, 16 to 32 μg/ml), or a low level of resistance (MIC, 4 to 8 μg/ml) or to be susceptible (MIC, <4 μg/ml) as described by Webber et al. (41). Isolates with MIC values between 0.125 and 2 μg/ml were considered to have decreased susceptibility. Three *S. enterica* serovar Typhimurium strains, L700, L701, and L702, were used as controls.

Detection of resistance determinants and isoelectric focusing. The isolates were analyzed to determine the presence of the following resistance genes related to their resistance phenotypes: $bla_{\text{PSE-1}}$, $bla_{\text{OXA-1}}$-like, $bla_{\text{TEM-1}}$-like, $bla_{\text{CTX-M}}$, and bla_{SHV} (encoding β-lactam resistance); *aadA1*-like, *aadA2*, *strA*, *aacC2*, *aacC4*, *aadB*, *armA*, *aphA1*, *aphA2*, and *aac*(*6*)*-1b* (aminoglycoside resistance); *catA1*, *cmlA1*, and *floR* (chloramphenicol resistance); *sul1*, *sul2*, and *sul3* (sulfamethoxazole resistance); *tet*(A), *tet*(B), and *tet*(G) (tetracycline resistance); *dfrA1*-like, *dfrA7-17*, *dfrA12*, *dfrA5-14*, and *dfrA17* (trimethoprim resistance); and *qnrA*, *qnrB*, *qnrS*, *qnrC*, and *qnrD* (decreased susceptibility to fluoroquinolone) (12, 21, 36). Mutations in the *gyrA* gene in the nalidixic acid-resistant isolates was determined by PCR sequencing, as previously described (20, 31). All isolates were screened for the presence of class 1 and 2 integrons by PCR sequencing using primer pairs 5′CS/3′CS (28) and Hep74/Hep51 (43), respectively. Sequencing was performed by AGOWA GmbH (Berlin, Germany), and the sequences obtained were compared with sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/GenBank/index.html).

For 19 ampicillin-resistant isolates (German turkey, turkey meat, and chicken isolates) the β-lactamase isoelectric point (pI) was determined by isoelectric focusing using a Phast System (GE Healthcare, Freiburg, Germany) as previously described (36).

Plasmid profile typing, macrorestriction analysis, and hybridization experiments. Bacterial plasmid DNA was obtained from the 55 German *S*. Saintpaul isolates (from turkey and turkey food products) by using the procedure of Kado and Liu (25) and was separated on 0.8% vertical agarose gels. *E. coli* reference plasmids R27 (169 kb), R1 (94 kb), RP4 (55 kb), and ColE1 (6 kb) were used as size standards.

Macrorestriction analysis of genomic DNA was carried out for all isolates using restriction endonucleases XbaI and BlnI (Roche Diagnostics, Mannheim, Germany). The fragments obtained were separated by pulsed-field gel electrophoresis (PFGE) using a CHEF-DRIII SYS220/240 system (Bio-Rad). Agarose gel plugs were prepared and PFGE was performed using the PulseNet standardized protocol (www.pulsenet-europe.org). The resulting profiles were analyzed by recording the presence or absence of fragments larger than 33 kb. Profiles in which there were differences in two or more bands were designated using numbers (e.g., X1, X2, etc., or B1, B2, etc.). Similar patterns in which only one band was different were designated using letters (e.g., X1, X1a, and X1b). The genetic similarity between profiles (XbaI, BlnI, or combined XbaI-BlnI PFGE profiles) was determined with the unweighted-pair method with arithmetic averages and Jaccard's coefficient, using the MVSP software (version 3.1; Multivariate Statistics Package for PCs; RockWare Inc., United States).

DNA from XbaI and BlnI PFGE and plasmid gels were transferred by Southern blotting onto membranes and hybridized with a probe for the *aadB* gene, using methods described previously (21). Plasmid gels were also hybridized with a $bla_{\text{TEM-1}}$ probe.

Statistical methods. To assess the discriminatory power of PFGE with XbaI and BlnI and a combination of the two methods, discrimination indices (DI) and their 95% confidence intervals (CI) were calculated using the Simpson's index of diversity as previously described (19, 24). This index of diversity determined the probability that two unrelated strains from the test population would be placed in different typing groups. The level of acceptance of discrimination of a typing method depends on several factors, and a DI greater than 0.9 is desirable.

RESULTS

Phenotypic antimicrobial resistance. The 55 *S.* Saintpaul isolates were tested to determine their resistance to several

TABLE 1. Sources and characteristics of German *S.* Saintpaul isolates (n = 55)

Group	Origin	No. of strains	Resistance phenotype[a]	Resistance genotype	Class 1 integron[b]	Origin (no. of isolates)[c]	PFGE profile			Plasmid sizes (no. of isolates)[d]
							XbaI	BlnI	No.	
1	Turkey baseline study feces samples (2006 and 2007)	16	[R][e]	[R′][f]	[I][g]	NI (13)	X1	B1	4	7 kb, two ≤6 kb (2); 7 kb, ≤6 kb; NP
							X1	B2	8	NP (7); 41 kb, 16 kb
							X1	B1b	1	28 kb
			[R]-TET	[R′]-*tet*(A)	[I]	MV	X1	B1	1	≤6 kb
						NI	X5	B2	1	NP
			[R]-TET	[R′]-*tet*(A)	[I] + 1,000 bp/*aadA1*	NI	X1	B2a	1	100 kb, 16 kb
2	Turkey diagnostic feces samples (2002 and 2003, 2005 and 2006)	17	[R]	[R′]	[I]	BW	X1	B1	1	16 kb
						BW, ST (3)	X1	B2	4	NP (3); 54 kb
						Unknown	X1a	B2	1	≤6 kb
						B	X14	B3	1	NP
						HE	X3	B10	1	≤6 kb
						B	X4	B4	1	16 kb
			[R]	[R′]	[I] + 1,000 bp/*aadA1*	B	X4	B4	1	100 kb, 49 kb
			[R]-TET	[R′]-*tet*(B)	[I]	NRW	X1	B2	1	47 kb, ≤6 kb
						BW, NI	X4	B4	2	49 kb, ≤6 kb; 62 kb, ≤6 kb
			[R]-CHL	[R′]-*sul3-cmlA*	[I]	SN	X1	B2	1	69 kb, 16 kb
			[R]-CHL-TMP/SXT	[R′]-bla_{PSE-1}-*sul3-cmlA*-(unknown)[h]	[I]	B	X2	B2c	1	100 kb, 16 kb
			Susceptible			BW (2)	X13a	B5	2	12 kb, three ≤6 kb (2)
3	Turkey-derived food diagnostic samples (2000 to 2007)	22	[R]	[R′]	[I]	NI	X1	B1	1	NP
						NRW	X1	B1a	1	NP
						NI, NRW	X1	B2	2	NP (2)
			[R][i]	[R′]	1,600 bp/*dfrA1-aadA1*	ST	X4	B4	1	≤6 kb
						NI	X4	B14	1	≤6 kb
			[R]	[R′]	[I] + 1,000 bp/*aadA1*	B	X4	B4	1	100 kb, 16 kb
			[R]-TET	[R′]-*aphA1-strA*-(unknown)[j]	[I]	B	X11	B11	1	138 kb, 70 kb, 9 kb, 7 kb, three ≤6 kb
			[R]-TET-TMP/SXT	[R′]-*aphA1-sul2-tet*(A)-*dfrA14*	[I]	B	X11	B11	1	174 kb, 77 kb, 37 kb
			[R]-CHL-TET-TMP/SXT	[R′]-*catA1-tet*(B)-*dfrA1*-like	[I]	BW, RP	X8	B2b	2	28 kb, ≤6 kb; ≤6 kb
			AMP-TET-STR/SPE-SUL-TMP/SXT	bla_{TEM-1}-*tet*(A)/*tet*(B)-*aadA1*-like-*sul1-dfrA1*-like	1,950 bp/*estX-aadA1* + 700 bp/*estX*	B	X6	B6	1	41 kb, three ≤6 kb
			TET-STR/SPE-SUL	*tet*(B)-*aadA1*-like-*sul1*	1,950 bp/*estX-aadA1* + 700 bp/*estX*	NRW	X6	B6	1	Three ≤6 kb
			TET-STR/SPE-SUL	*tet*(B)-*aadA1*-like-*sul1*	1,950 bp/*estX-aadA1*	B (2)	X6	B6	2	Three ≤6 kb; 70 kb, three ≤6 kb
			AMP-CHL-TET-STR-SUL-TMP/SXT	bla_{TEM-1}- *catA1-tet*(A)-*strA*/*aadA1*-like-*sul1*/*sul2-dfrA1*-like	1,600 bp/*dfrA1-aadA1*	SH	X10	B8	1	NP
			AMP/AMC(i/r)-NAL-TET-STR-SUL	bla_{TEM-1}-$gyrA_{Ser83→Tyr83}$-*tet*(A)-*strA-sul2*	200 bp/without gene cassettes	NRW	X7	B7	1	100 kb, two ≤6 kb
			NAL	$gyrA_{Ser83→Tyr83}$		BW	X7a	B7	1	16 kb, two ≤6 kb
			GEN-NAL-TET-STR/SPE-SUL	$gyrA_{Ser83→Glu83}$-*tet*(B)-*aadA1*-like-*sul1*	1,000 bp/*aadA1*	TH	X9	B9	1	138 kb, 70 kb
			Susceptible			RP	X13a	B5	1	12 kb, three ≤6 kb
						NRW	X12	B12	1	74 kb
						SN	X13	B13	1	Two ≤6 kb

[a] Resistance phenotypes were assessed by the broth microdilution method as described by Schroeter et al. (37) by following the guidelines of the CLSI (13). The breakpoints used for ampicillin (AMP), amoxicillin-clavulanic acid (AMC), ceftiofur (XNL), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), sulfonamides (SUL), and tetracycline (TET) were the CLSI breakpoints (14). For ciprofloxacin, the MIC breakpoints were as follows: resistant, ≥4 μg/ml; intermediate, 2 μg/ml; and susceptible, ≤1 μg/ml (decreased susceptibility, 1 to 0.12 μg/ml; fully susceptible, ≤0.06 μg/ml). The breakpoints used for colistin (COL) (resistant, ≥16 μg/ml; susceptible, ≤8 μg/ml), florfenicol (FLO) (resistant, ≥32 μg/ml; susceptible, ≤8 μg/ml), nalidixic acid (NAL) (resistant, ≥32 μg/ml; susceptible, ≤16 μg/ml), spectinomycin (SPE) (resistant, ≥128 μg/ml; susceptible, ≤64 μg/ml), and trimethoprim (TMP) (resistant, ≥16 μg/ml; susceptible, ≤8 μg/ml) were the DANMAP breakpoints (17). And the breakpoints used for neomycin (NEO) (resistant, ≥16 μg/ml; susceptible, ≤4 μg/ml), streptomycin (STR) (resistant, ≥32 μg/ml; susceptible, ≤8 μg/ml), and trimethoprim-sulfamethoxazole (SXT) (resistant, ≥4 and ≥76 μg/ml respectively; susceptible, ≤2 and ≤38 μg/ml, respectively) were the breakpoints of Schroeter et al. (37).

[b] Variable regions of class 1 integrons; indicated are their lengths (determined using the 5′CS and 3′CS primers [28]) and the inserted gene cassettes.

[c] B, Berlin; BW, Baden-Württemberg; HE, Hesse; MV, Mecklenburg Western Pomerania; NI, Lower Saxony; NRW, North Rhine-Westphalia; RP, Rhineland-Palatinate; SH, Schleswig-Holstein; SN, Saxony; ST, Saxony-Anhalt; TH, Thuringia. Unless indicated otherwise, the number of isolates was one.

[d] NP, no plasmid detected. Unless indicated otherwise, the number of isolates was one.

[e] [R], core resistance phenotype [AMP/AMC(i/r)-GEN(i/r)-KAN(i/r)-NAL-CIP(i/ds)-STR/SPE-SUL], where (i/r) indicates intermediate or full resistance and (i/ds) indicates intermediate or decreased susceptibility.

[f] [R′], core resistance genotype [bla_{TEM-1}-*aadB*-$gyrA_{Ser83→Glu83}$-*aadA1*-like/*aadA2-sul1*].

[g] [I], class 1 integron with the 1,700 bp/*aadB-aadA2* variable region.

[h] None of the genes encoding trimethoprim resistance tested were detected in the strain.

[i] The strain exhibited the core resistance pattern, but the class 1 integron with the 1,700 bp/*aadB-aadA2* variable region was not detected by PCR.

[j] None of the genes encoding tetracycline resistance tested were detected in the strain.

TABLE 2. Resistance to individual antibiotics and prevalence of selected resistance genes in German *S*. Saintpaul isolates ($n = 55$)[a]

Antimicrobial[b]	No. (%) of resistant isolates	Resistance gene(s)	Isolates containing resistance gene(s)	
			No. (%)	Origin (no. of isolates)[c]
β-Lactams				
Ampicillin	45 (82)	bla_{PSE-1}	1 (2)	G2 (1)
		bla_{OXA-1}-like	0	
		bla_{TEM-1}-like	45 (100)	G1 (16), G2 (15), G3 (14)
		bla_{CTX-M}	0	
		bla_{SHV}	0	
Aminoglycosides				
Streptomycin	49 (89)	*strA*	3 (6)	G3 (3)
		aadA1-like	48 (98)	G1 (16), G2 (15), G3 (17)
		aadA2	42 (86)	G1 (16), G2 (15), G3 (11)
Gentamicin	43 (78)[d]	*aacC2*	0	
		aacC4	0	
		aadB[e]	42 (98)	G1 (16), G2 (15), G3 (11)
		armA	0	
		Others[f]	1 (2)	G3 (1)
Kanamycin	42 (76)[d]	*aphA1*	2 (5)	G3 (2)
		aphA2	0	
		aac(6)-1b	0	
Phenicols				
Chloramphenicol	5 (9)	*catA1*	3 (3/5)[g]	G3 (3)
		cmlA1	2 (2/5)[g]	G2 (2)
		floR	0	
Folate pathway inhibitors				
Sulfamethoxazole	49 (89)	*sul1*	48 (98)	G1 (16), G2 (15), G3 (17)
		sul2	3 (6)	G3 (3)
		sul3	2 (4)	G2 (2)
Trimethoprim	6 (11)	*dfrA1*-like	4 (4/6)[g]	G3 (4)
		dfrA7	0	
		dfrA12	0	
		dfrA14	1 (1/6)[g]	G3 (1)
		dfrA17	0	
		Others[f]	1 (1/6)[g]	G2 (1)
Tetracyclines				
Tetracycline	17 (31)	*tet*(A)	5 (29)	G1 (1), G3 (4)
		tet(B)	10 (59)	G2 (3), G3 (7)
		tet(G)	0	
		Others[f]	3 (18)	G1 (2), G3 (1)
Quinolones and fluoroquinolones				
Nalidixic acid and ciprofloxacin	45 (82), 16 (29)[h]	*qnrA*	0	
		qnrB	0	
		qnrC	0	
		qnrD	0	
		qnrS	0	

[a] Five of the 55 isolates selected were susceptible to all of the antimicrobials tested.
[b] The breakpoints used are shown in Table 1. Seven other antimicrobials were also tested: no resistance to florfenicol, ceftiofur, or colistin was detected; 46 (84%) of the isolates were resistant to spectinomycin; 6 (11%) of the isolates were resistant to trimethoprim-sulfamethoxazole; 13 (24%) of the isolates were resistant to amoxicillin-clavulanic acid; and 2 (4%) of the isolates were resistant to neomycin.
[c] G1, turkey baseline study (feces) performed in 2006 and 2007; G2, turkey diagnostic isolates (feces) collected in 2002 and 2003 and in 2005 and 2006; G3, turkey-derived food diagnostic isolates collected in 2000 to 2007.
[d] Intermediate or full resistance.
[e] The *aadB* gene also confers a low resistance to kanamycin according to Zhao et al. (45).
[f] The resistance mechanisms were not identified in this study.
[g] Because of the low number of resistant isolates (<10), only the absolute number is indicated.
[h] For ciprofloxacin, only isolates with intermediate resistance (MIC, 2 μg/ml) are included. An additional 29 isolates (53%) showed reduced susceptibility (MIC, 1 to 0.25 μg/ml).

β-lactams by using agar diffusion and/or disk diffusion. The results and corresponding breakpoints are shown in Table 3. Eighty-two percent of the isolates showed resistance to penicillins, 75% of the isolates showed resistance to cephalothin, and 64% of the isolates showed resistance to cefuroxime (an additional 36% of the isolates showed intermediate or decreased susceptibility to this antimicrobial agent). No isolate showed full resistance to the different third-generation cephalosporins, but intermediate resistance was detected (14.5% of the isolates showed intermediate resistance to cefoxitin, 34.5%

TABLE 3. β-Lactam susceptibility tests with *S.* Saintpaul isolates ($n = 55$)

Susceptibility test[a]	Antimicrobial agents		Concn tested	No. (%) of isolates				
	Subclass	Antibiotic		Resistant	Intermediate	Susceptible[b]	Decreased susceptibility	Fully susceptible
Agar dilution (MIC)	First-generation cephalosporins	Cephalothin	1–32 μg/ml	41 (74.5)	4 (7.3)		10 (18.2)	
	Second-generation cephalosporins	Cefuroxime	1–32 μg/ml	35 (63.6)	10 (18.2)		10 (18.2)	
	Third-generation cephalosporins	Cefotaxime	1–64 μg/ml					55 (100)
		Ceftazidime	1–32 μg/ml				5 (9.1)	50 (90.1)
		Cefoxitin	1–32 μg/ml		8 (14.5)		40 (72.7)	7 (12.7)
		Ceftiofur	0.5–8 μg/ml		19 (34.5)		31 (56.4)	5 (9.1)
Disk diffusion (zone diam)	Penicillins	Ampicillin	10 μg	45 (81.8)		10 (18.2)		
	Carboxypenicillins	Ticarcillin	75 μg	45 (81.8)		10 (18.2)		
	Acylaminopenicillins	Piperacillin	100 μg	45 (81.8)		10 (18.2)		
	β-Lactamase inhibitors	Amoxicillin-clavulanic acid	30 μg	13 (23.6)	27 (49.1)	15 (27.3)		
	Third-generation cephalosporins	Cefpodoxime	10 μg		6 (10.9)	49 (89.1)		
		Ceftriaxone	30 μg			55 (100)		
	Fourth-generation cephalosporins	Cefepime	30 μg			55 (100)		
	Carbapenems	Imipenem	10 μg			55 (100)		
	Monobactams	Aztreonam	30 μg			55 (100)		

[a] The MIC breakpoints and zone diameter standards were defined as described by the CLSI (16). The MIC breakpoints for cephalothin, cefuroxime, ceftazidime, and cefoxitin were as follows: resistant, ≥32 μg/ml; intermediate, 16 μg/ml; and susceptible, ≤8 μg/ml (decreased susceptibility, 8 to 2 μg/ml; fully susceptible, <2 μg/ml). The MIC breakpoints for cefotaxime were as follows: resistant, ≥64 μg/ml; intermediate, 32 to 16 μg/ml; and susceptible, ≤8 μg/ml (decreased susceptibility, 8 to 2 μg/ml; fully susceptible, <2 μg/ml). The MIC breakpoints for ceftiofur were as follows: resistant, ≥8 μg/ml; intermediate, 4 μg/ml; and susceptible, <4 μg/ml (decreased susceptibility, 2 to 1 μg/ml; fully susceptible, <1 μg/ml). The zone diameter standards for cefpodoxime and piperacillin were as follows: resistant, ≤17 mm; intermediate, 18 to 20 mm; and susceptible, ≥21 mm. The zone diameter standards for ampicillin were as follows: resistant, ≤13 mm; intermediate, 14 to 16 mm; and susceptible, ≥17 mm. The zone diameter standards for ceftriaxone were as follows: resistant, ≤13 mm; intermediate, 14 to 20 mm; and susceptible, ≥21 mm. The zone diameter standards for amoxicillin-clavulanic acid were as follows: resistant, ≤13 mm; intermediate, 14 to 17 mm; and susceptible, ≥18 mm. The zone diameter standards for cefepime were as follows: resistant, ≤14 mm; intermediate, 15 to 17 mm; and susceptible, ≥18 mm. The zone diameter standards for ticarcillin were as follows: resistant, ≤14 mm; intermediate, 15 to 19 mm; and susceptible, ≥20 mm. The zone diameter standards for imipenem were as follows: resistant, ≤13 mm; intermediate, 14 to 15 mm; and susceptible, ≥16 mm. And the zone diameter standards for aztreonam were as follows: resistant, ≤15 mm; intermediate, 16 to 21 mm; and susceptible, ≥22 mm.

[b] Includes isolates with decreased and full susceptibility.

of the isolates showed intermediate resistance to ceftiofur, and 11% of the isolates showed intermediate resistance to cefpodoxime), as was decreased susceptibility (73% of the isolates showed decreased susceptibility to cefoxitin, 56% of the isolates showed decreased susceptibility to ceftiofur, and 9% of the isolates showed decreased susceptibility to ceftazidime). The double-disk synergy test using different third-generation cephalosporins and clavulanic acid was negative for all isolates.

The 16 isolates in group 1 (from the *Salmonella* baseline study of turkeys) showed two different antimicrobial resistance profiles with the core resistance pattern, AMP/AMC(i/r)-GEN(i/r)-KAN(i/r)-NAL-CIP(i/ds)-STR/SPE-SUL (Table 1), which includes full resistance to ampicillin, streptomycin/spectinomycin, sulfamethoxazole, and nalidixic acid, full (r) or intermediate (i) resistance to amoxicillin-clavulanic acid (MIC, 16 to 32 μg/ml), gentamicin (MIC, 8 to 32 μg/ml), and kanamycin (MIC, 32 to 64 μg/ml), intermediate or decreased susceptibility (ds) to ciprofloxacin (MIC, 2 or 1 μg/ml, respectively), and intermediate or decreased susceptibility to ceftiofur (MIC, 4 to 2 μg/ml). This pattern was also the pattern most frequently observed for groups 2 (diagnostic feces isolates) and 3 (diagnostic food isolates). In addition, this core resistance pattern was also connected with other resistance patterns. In group 3 five other resistance phenotypes were also detected (Table 1). This core resistance pattern was also found in 15 of the 24 outgroup isolates (German chicken and human isolates and Dutch poultry and poultry food isolates).

Determination of resistance to organic solvents and disinfectants. *E. coli* K-12 wild-type control strain AG100 produced confluent growth with *n*-hexane and did not grow in the presence of cyclohexane, whereas *E. coli* AG102 (resistant control) grew in the presence of both *n*-hexane and cyclohexane (42). All 55 *S.* Saintpaul isolates were tolerant to *n*-hexane, whereas 43 of them (78%) exhibited tolerance to cyclohexane. Furthermore, all isolates (including the outgroup isolates) were susceptible to triclosan, determined as described by Webber et al. (41), and the MIC values were 0.06 μg/ml (13 isolates), 0.125 μg/ml (8 isolates), 0.25 μg/ml (50 isolates), 0.5 μg/ml (7 isolates), and 1 μg/ml (1 isolate).

Antimicrobial resistance determinants. The prevalence of resistance genes and their distribution in the different groups are shown in Table 2. The $bla_{\text{TEM-1}}$-like, *aadB*, *aadA1*-like, *aadA2*, and *sul1* genes conferring resistance to ampicillin, gentamicin and kanamycin, streptomycin and spectinomycin, and sulfamethoxazole, respectively, were the genes that were found most frequently. The *aadB* gene was detected in 42 of 43 isolates with intermediate or full resistance to gentamicin. Forty of these 42 isolates exhibited intermediate or low resistance to kanamycin (MIC for 36 isolates, 32 μg/ml; MIC for 4 isolates, 64 μg/ml), and 2 isolates, which also contained the

aphA1 gene, exhibited full resistance to kanamycin (MIC, >64 μg/ml).

All 45 ampicillin-resistant isolates (82% of the study group) (Tables 2 and 3) contained bla_{TEM-1}-like genes (these genes were not present in the ampicillin-susceptible isolates). The isoelectric focusing method revealed the presence of a β-lactamase with an isoelectric point (pI) of 5.4 (characteristic of TEM-1) in the 19 ampicillin-resistant *S.* Saintpaul isolates tested. Only one of these isolates also produced a band at approximately pI 5.7, which is characteristic of PSE-1. Sequencing of the bla_{TEM-1}-like PCR products confirmed the presence of the bla_{TEM-1} gene.

As shown in Table 2, 45 of 55 (82%) isolates were resistant to nalidixic acid (MIC, >128 μg/ml) and showed intermediate or decreased susceptibility to ciprofloxacin (MIC, 2 to 0.25 μg/ml). The other 10 isolates were susceptible to nalidixic acid (MIC, ≤8 μg/ml), and the MIC of ciprofloxacin were ≤0.03 μg/ml. Sequence analysis revealed that in all 45 nalidixic acid-resistant isolates there was a point mutation in the *gyrA* gene (resulting in a change from Ser83 to Glu83 in 43 isolates [96%] and in a change from Ser83 to Thr83 in 2 food isolates [4%]). All 55 isolates were negative for the plasmid quinolone resistance genes *qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*.

Forty-nine isolates contained class 1 integrons. Six types of class 1 integrons were identified based on the amplicon size and the gene cassette content in the variable region (Table 1). In 37 isolates (64% of the series) class 1 integrons with a 1,700-kb variable region contained *aadB-aadA2* gene cassettes. All isolates containing class 1 integrons were also positive for *qacEΔ1* (conferring resistance to quaternary ammonium compounds) and, with the exception of one isolate (with a variable-region amplicon consisting of only 200 bp), for *sul1*. Class 2 integrons were not detected.

To locate the 1,700-bp class 1 integron, PFGE and plasmid DNA Southern blot hybridization using an *aadB* probe were performed. The *aadB*-containing integron mapped on chromosomal XbaI fragments ranging from 150 to 245 kb long. In all isolates that produced PFGE patterns similar to the X1 pattern described below (Fig. 1), the integron was always inserted into the smaller, ~150-kb fragment. The same hybridization experiment was performed with BlnI-digested chromosomal DNA, and it showed that *aadB* was in fragments ranging from 470 to 600 kb long. Plasmid DNA hybridization with *aadB* or bla_{TEM-1} was negative.

Molecular typing. Thirty-seven (67%) of the isolates harbored plasmids (26 different patterns, with one to seven plasmids ranging from 6 to 174 kb) (Table 1). Only 13 isolates (seven of them from food) harbored plasmids larger than 50 kb. The other 18 isolates (33%), one-half of them (9 isolates) originating from the turkey baseline study (group 1), did not harbor any plasmid.

As determined by PFGE typing, the 55 isolates exhibited 17 XbaI patterns (DI, 0.76 [CI, 0.64 to 0.87]), 19 BlnI patterns (DI, 0.86 [CI, 0.80 to 0.93]), and 22 combined XbaI-BlnI patterns (DI, 0.89 [CI, 0.83 to 0.95]) (Fig. 1 to 3). Eleven XbaI profiles and 12 BlnI profiles were represented by only one isolate, whereas all other profiles were represented by at least two isolates. For the baseline study isolates (group 1) two XbaI patterns and four BlnI patterns were found. For the diagnostic fecal isolates (group 2) the two enzymes each produced seven patterns. The greatest heterogeneity was found for the food isolates (group 3), which exhibited 12 XbaI patterns and 14 BlnI patterns. Information about PFGE patterns, groups, and isolation regions and years is shown in Fig. 1 to 3 and Table 1. The XbaI profile detected most frequently was profile X1 (26 isolates [47%]), which was produced by 15, 7, and 4 isolates belonging to groups 1, 2, and 3, respectively. The most frequent profiles obtained by BlnI digestion were profiles B2 (18 isolates) and B1 (8 isolates). Profile B2 was detected for 9, 7, and 2 isolates belonging to groups 1, 2, and 3, respectively.

Based on the XbaI analysis, a cluster that included the X1 and X1a profiles (93% similarity; 27 isolates, including 15 isolates belonging to group 1, 8 isolates belonging to group 2, and 4 isolates belonging to group 3) was recognized (Fig. 1). As determined by BlnI analysis, 7 BlnI profiles (B1, B1a, B1b, B2, B2a, B2b, and B2c) clustered together (75% similarity; 31 isolates, including 16 isolates belonging to group 1, 10 isolates belonging to group 2, and 5 isolates belonging to group 3) (Fig. 2). There was a clonal relationship among the 27 isolates that clustered together at 90% similarity using the combinations of profiles (profiles X1 and X1a combined with profiles B1, B1a, B1b, B2, and B2a; 15 isolates belonging to group 1, 8 isolates belonging to group 2, and 4 isolates belonging to group 3) or, as shown above, at 93% similarity using the XbaI profiles (Fig. 1).

The outgroup isolates were also analyzed by PFGE with XbaI. Profile X1 was detected for one Dutch food isolate and two German human isolates. Several of the other profiles found previously were also detected for these isolates, including profile X8 for both German chicken isolates and 10 Dutch poultry isolates, profile X4 for one Dutch poultry isolate, profile X13a for three German human isolates, and profile X6 for one German human isolate.

DISCUSSION

Recently, *S.* Saintpaul has become more important, and in 2006, it ranked among the top five causes of human salmonellosis in Europe (http://ecdc.europa.eu/en/publications/Pages/Surveillance_Reports.aspx). This development has been noticed not only in European countries but also worldwide. Exceptionally eye-catching were two multistate *S.* Saintpaul outbreaks, one in 2006 in Australia that was associated with cantaloupe consumption (34) and the other in the United States that affected around 1,500 people in the summer of 2008 and was due to vegetables such as jalapeño peppers and tomatoes (4). The latest outbreak, in February 2009 in the United States, was associated with alfalfa sprouts (6). In Germany an important nationwide outbreak in 1993 was linked to contaminated paprika (27), and the isolates recovered showed a high degree of similarity to strains isolated from turkeys, which were suspected to have caused sporadic human infections (9). Most of the outbreaks reported to date in which *S.* Saintpaul isolates were implicated were related to the consumption of vegetables (paprika, tomatoes, alfalfa) and to the consumption of turkey products (i.e., ready-to-eat meat products) (22, 26), indicating that both kinds of foods have important roles as vehicles of infection. Whereas antimicrobial agent-susceptible strains were involved in most of the vegetable-related outbreaks, the

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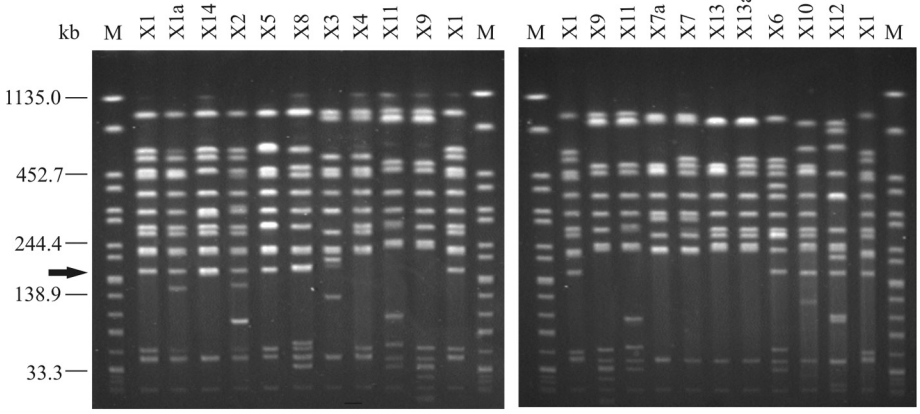

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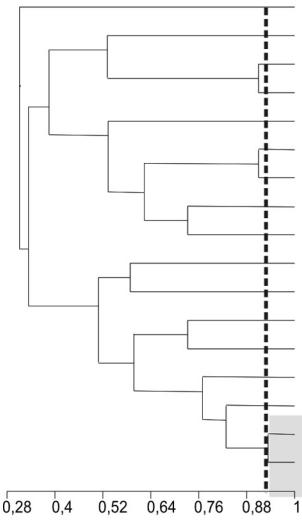


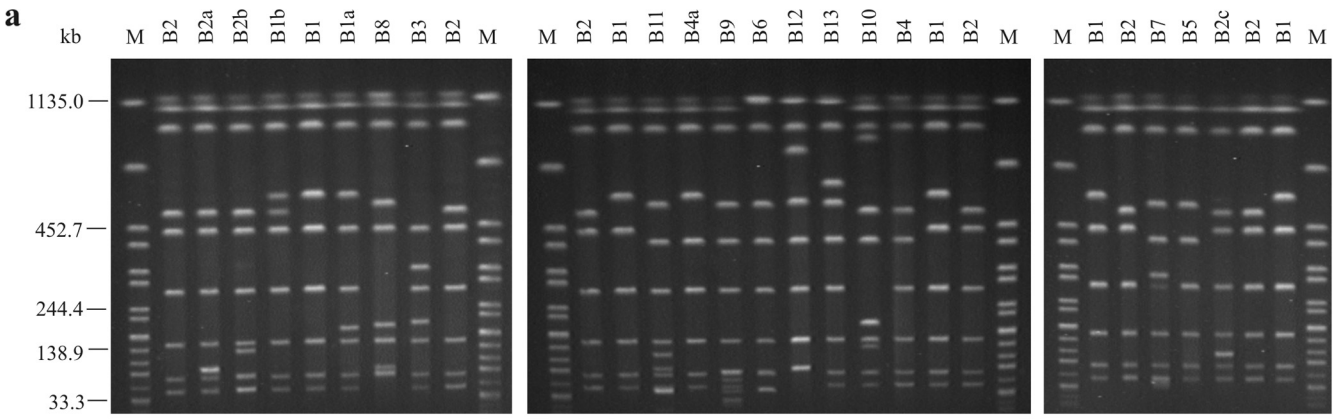
XbaI Pattern (No.)	BlnI Pattern (No.)	Group (No.)	Isolation Year (No.)	Isolation Region (No.)	Reference Strain
X12	B12	G3	2006	NRW	06-01242
X6 (4)	B6 (4)	G3 (4)	2007 (4)	B (3), NRW	07-00334
X7	B7	G3	2007	NRW	07-01360
X7a	B7	G3	2007	BW	07-00039
X10	B8	G3	2000	SH	00-03122
X13a (3)	B5 (3)	G2 (2), G3	2005 (2), 2006	BW (2), RP	05-01959
X13	B13	G3	2007	SN	07-02022
X9	B9	G3	2007	TH	07-02208
X11 (2)	B11 (2)	G3 (2)	2000, 2006	B (2)	00-02151
X8 (2)	B2b (2)	G3 (2)	2004, 2006	BW, RP	06-04383
X5	B2	G2	2003	B	07-00303
X4 (7)	B4 (6), B14	G2 (4), G3 (3)	2002, 2003 (2), 2006 (3), 2007	ST, B (3), BW, NI (2)	06-01828
X3	B10	G2	2006	HE	06-01822
X2	B2c	G1	2007	NI	03-01845
X14	B3	G2	2003	B	03-00314
X1a	B2	G2	2003	unknown	03-01331
X1 (26)	B1 (7), B1a, B1b, B2 (16), B2a	G1 (15), G2 (7), G3 (4)	2003 (7), 2005, 2006 (4), 2007 (14)	NI (15), MV (2), BW (2), NRW (3), SN, ST (3)	06-04821

FIG. 1. (a) XbaI PFGE profiles of representative *S.* Saintpaul isolates. To define the profiles, only bands at >33 kb were considered. Similar profiles with only a one-band difference were designated by letters. Profiles with differences in two or more bands were designated by numbers. Profiles X1 and X1a are the profiles of the clonal line. The arrow indicates the position of the *aadB* gene. Lanes M contained XbaI-digested DNA of *S. enterica* serovar Braenderup H9812, which was used as a size standard. (b) Dendrogram showing the genetic similarity between XbaI profiles determined by using the unweighted-pair group method with arithmetic averages (UPGMA) and Jaccard's coefficient (*J*). The numbers in parentheses are the numbers of isolates when there was more than one isolate. G1, group 1 (baseline study); G2, group 2 (diagnostic feces study); G3, group 3 (diagnostic food study). Abbreviations for isolation regions in Germany are explained in Table 1. The shading indicates related clonal patterns at a Jaccard's coefficient of 0.93.

isolates obtained from turkey-related outbreaks were multidrug resistant.

The European Food Safety Authority report on the baseline survey in 2006 and 2007 on the prevalence of *Salmonella* in turkey flocks indicated that *S.* Saintpaul was one of the most frequent serotypes (10.4% of isolates) in positive flocks of fattening turkeys in Europe and the leading serotype in Poland, Slovakia, and Netherlands (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178706574172.htm). In Germany 98 flocks of breeding turkeys and 295 flocks of fattening turkey were sampled, and 111 *Salmonella* isolates were obtained. Nineteen (17%) of these isolates were *S.* Saintpaul strains, and this serovar was the third most common serovar (5). All *S.* Saintpaul isolates collected in the study showed resistance to at least five antimicrobials and were included (except for siblings) in the present study.

Of the 52,318 *Salmonella* isolates typed at NRL-Salm from 1998 to September 2009, 799 were *S.* Saintpaul isolates, and 533 (67%) of these *S.* Saintpaul isolates originated from turkeys or turkey products. More than 90% of these isolates were resistant to 2 to 12 antimicrobials. Similar results were described by other workers (33, 35). The most common types of resistance found for these *S.* Saintpaul isolates were resistance to ampicillin (85% of the isolates), resistance to sulfamethoxazole (82%), resistance to nalidixic acid (79%), and resistance to streptomycin (75%); 34% and 25% of the isolates were resistant to gentamicin and kanamycin, whereas 44% and 49% of the isolates exhibited in-



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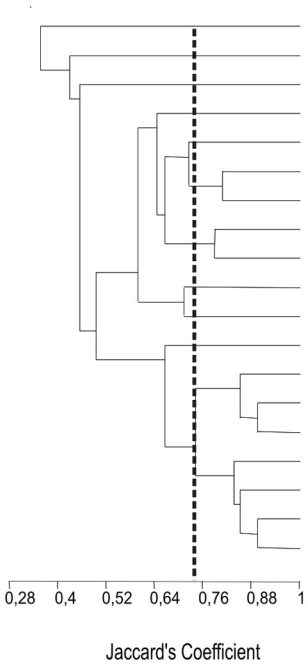

BlnI Pattern (No.)	XbaI Pattern (No.)	Group (No.)	Isolation Year (No.)	Isolation Region (No.)	Reference Strain
B12	X12	G3	2006	NRW	06-01242
B8	X10	G3	2000	SH	00-03122
B10	X3	G2	2006	HE	06-01822
B13	X13	G3	2007	SN	07-02022
B7 (2)	X7, X7a	G3 (2)	2007 (2)	NRW, BW	07-01360
B5 (3)	X13a (3)	G3 (2), G3	2005 (2), 2006	BW (2), RP	05-01959
B9	X9	G3	2007	TH	07-02208
B4 (6)	X4 (6)	G2 (4), G3 (2)	2002, 2003 (2), 2006 (3)	ST, B (3), BW, NI	06-01828
B14	X4	G3	2007	NI	07-02593
B6 (4)	X6 (4)	G3 (4)	2007 (4)	B (3), NRW	07-00334
B11 (2)	X11 (2)	G3 (2)	2000, 2006	B (2)	00-02151
B3	X14	G2	2003	B	03-00314
B1a	X1	G3	2006	NRW	06-01775
B1 (7)	X1 (7)	G1 (5), G2, G3	2003, 2006 (3), 2007 (3)	NI (4), MV (2), BW	06-04821
B1b	X1	G1	2007	NI	07-00648
B2c	X2	G1	2007	NI	03-01845
B2b (2)	X8 (2)	G3 (2)	2004, 2006	BW, RP	06-004383
B2a	X1	G1	2007	NI	07-00301
B2 (18)	X1 (16), X1a, X5	G1 (8), G2 (8), G3 (2)	2003 (8), 2005, 2007 (9)	NI (9), NRW (2), SN, ST (3), BW, B, unknown	07-00297

FIG. 2. (a) BlnI PFGE profiles of representative *S.* Saintpaul isolates. To define profiles, only bands at >33 kb were considered. Similar profiles with only a one-band difference were designated by letters. Profiles with differences in two or more bands were designated by numbers. Profiles B1, B1a, B1b, B2, and B2a are the profiles of the clonal line. Lane M contained XbaI-digested DNA of *S. enterica* serovar Braenderup H9812, which was used as a size standard. (b) Dendrogram showing the genetic similarity between BlnI profiles determined by using the unweighted-pair group method with arithmetic averages (UPGMA) and Jaccard's coefficient (*J*). The numbers in parentheses are the numbers of isolates when there was more than one isolate. G1, group 1 (baseline study); G2, group 2 (diagnostic feces study); G3, group 3 (diagnostic food study). Abbreviations for isolation regions in Germany are explained in Table 1. The B1, B1a, B1b, B2, B2a B2b, and B2c profiles clustered together at a Jaccard's coefficient of 0.75.

termediate resistance to these antimicrobials. When the strains used in the study were selected, the intention was to use representative isolates obtained from active and passive monitoring over a defined period of time and to compare them to human and other European isolates. As demonstrated in the present study and in previous studies (32), some of the types of resistance observed for *S.* Saintpaul isolates are related to the presence of class 1 integrons (Table 1), which are genetic structures that confer multidrug resistance. The presence of integrons enhances the coselection of different types of resistance.

For the organisms analyzed in this study, the percentage of isolates resistant to nalidixic acid in both feces and food samples was also high (82%). The percentage of nalidixic acid-resistant isolates was much higher than the percentages found in other poultry studies (7, 11, 30, 31). These isolates displayed intermediate resistance to ciprofloxacin (16 isolates [29%] with an MIC of 2 μg/ml) or decreased susceptibility to this agent (25 isolates [46%] with an MIC of 1 μg/ml and 4 isolates [7%] with MIC of 0.5 to 0.25 μg/ml). Using the harmonized cutoff values defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (www.eucast.org), as required in

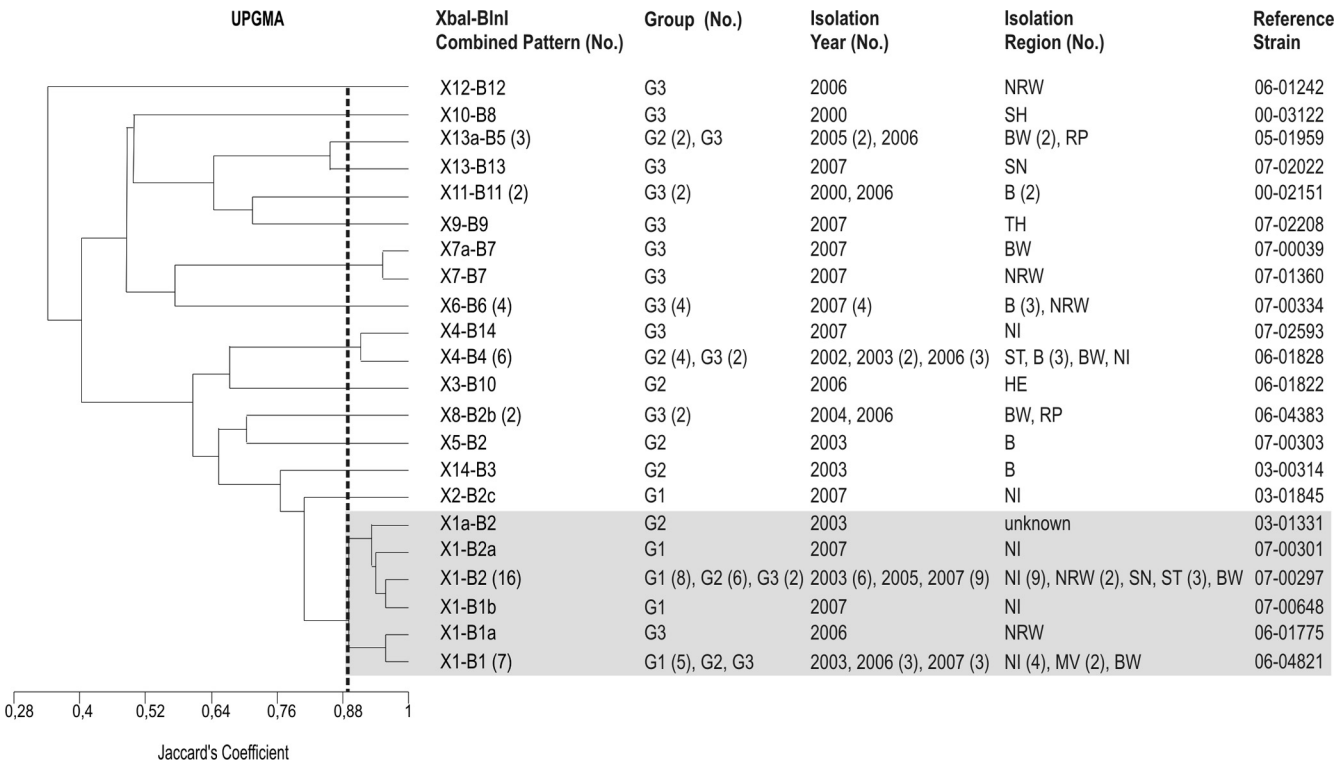

FIG. 3. Dendrogram showing the genetic similarity between combined XbaI-BlnI profiles determined by using the unweighted-pair group method with arithmetic averages (UPGMA) and Jaccard's coefficient (*J*). The numbers in parentheses are the numbers of isolates when there was more than one isolate. G1, group 1 (baseline study); G2, group 2 (diagnostic feces study); G3, group 3 (diagnostic food study). Abbreviations for isolation regions in Germany are explained in Table 1. The shading indicates related clonal patterns at a Jaccard's coefficient of 0.90.

the EU guidelines for monitoring antimicrobial resistance (10), all these isolates are now considered resistant (cutoff value, >0.06 μg/ml). Because of this, the results are worrying since fluoroquinolones are antimicrobial agents that are critically important in human and veterinary medicine (3). As described by Malorny et al. (31), nalidixic acid-resistant isolates were isolated more frequently from turkeys than from other poultry. Although *qnr* genes were found in *S*. Saintpaul isolates analyzed in other studies (12, 18; B. Guerra, unpublished data), no *qnrA*, *qnrB*, *qnrS*, *qnrC*, or *qnrD* genes were detected in the 55 isolates tested. However, these isolates had mutations in the quinolone resistance-determining region of the *gyrA* gene and probably had enhanced active efflux mechanisms that could be implicated in the resistance to cyclohexane and the decreased susceptibility to triclosan and fluoroquinolones (29, 41, 42).

A common core resistance pattern, AMP/AMC(i/r)-GEN(i/r)-KAN(i/r)-NAL-CIP(i/ds)-STR/SPE-SUL, was found preferentially in the isolates from feces samples. This pattern was associated with intermediate resistance or decreased susceptibility to ciprofloxacin, resistance to first-generation cephalosporins, resistance or intermediate resistance to second-generation cephalosporins, and intermediate or decreased susceptibility to some third-generation cephalosporins. From a public health perspective, this finding is also worrisome, because these antimicrobials, together with the fluoroquinolones, are drugs of choice for clinical treatment of systemic *Salmonella* infections (3, 23). However, the results of screening for resistance-conferring β-lactam genes, such as $bla_{\text{CTX-M}}$, bla_{SHV}, and $bla_{\text{OXA-1}}$-like, were negative, and only the $bla_{\text{TEM-1}}$ gene, located in the chromosome, was detected. Therefore, we speculate that the negative isolates possess other molecular mechanisms (e.g., loss of porins, genes that were not detected, or multidrug resistance pumps) (38) that are responsible for resistance to β-lactams, which could not be determined in the present work. In order to examine this possibility, these isolates are being investigated further.

PFGE typing proved that 15 of 16 isolates used in the baseline survey (isolated from five farms in two federal regions) exhibiting the core resistance pattern described above belonged to the same clonal line (XbaI PFGE patterns with 93% genetic similarity). All of the isolates belonging to this clonal line had the same core resistance genotype ($bla_{\text{TEM-1}}$, *aadB*, *aadA2*, *sul1*, and the Ser83→Glu83 mutation in the *gyrA* gene), including a class 1 integron conferring resistance to gentamicin, kanamycin, streptomycin/spectinomycin, and sulfonamides. Overall, the distribution of plasmids in *S*. Saintpaul was highly variable, although the majority of isolates that belonged to the clonal line contained only small plasmids or no plasmids. In fact, hybridization experiments showed that the class 1 integron carrying the *aadB* gene was located on the chromosome. This integron, which was also present in 9 other isolates included in our work that do not belong to the same clonal line, was described in previous studies (32, 45), but to our knowledge, so far the *aadB* gene has been detected only on plasmids in *Salmonella* (39) and a chromosomal location has not been described previously. In some of the poultry- and turkey-rearing facilities where the *S.* Saintpaul strains were

isolated, antimicrobials like penicillin and enrofloxacin were used (unpublished data). Consequently, the presence of chromosomally located resistance determinants (i.e., genes and mutations) in the *S*. Saintpaul isolates conferred a selective advantage in the presence of the antimicrobial selection pressure on these farms.

Derivatives of the multiresistant *S*. Sainpaul strains described above were found throughout Germany not only among isolates examined in the baseline study performed in 2006 and 2007 but also in diagnostic feces samples obtained previously in 2003 to 2005. Isolates belonging to this clonal line were also found among *S.* Saintpaul isolates that originated from poultry in neighboring countries, like Netherlands, which suggests that this line might be widespread. This clonal line was also represented by German food isolates, making transmission to humans likely. The analysis of human isolates provided by the Robert Koch Institute confirmed this hypothesis. The results presented here describe the characteristics of multiresistant *S*. Saintpaul isolates originating from turkeys whose spread and molecular development should be examined further for protection of public health.

ACKNOWLEDGMENTS

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