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Incidence of the Recently Described Sulfonamide Resistance Gene *sul3* among German *Salmonella enterica* Strains Isolated from Livestock and Food

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The *sul3* gene recently described in *Escherichia coli* was found in 22 of 512 (4.3%) German *Salmonella* isolates from different regions and sources and of different serotypes, antimicrobial resistance phenotypes, and genomic groups. This is the first report on the prevalence of *sul3* among *Salmonella* strains, and the findings support the strong potential of this determinant to spread within bacterial populations.

Sulfonamides represent the oldest group of antimicrobial agents and have been available since the 1930s. They competitively inhibit the bacterial enzyme dihydropteroic acid synthetase (DHPS). Resistance to sulfonamides appeared quite soon after their introduction into clinical practice. Resistance can result from mutations in the chromosomal DHPS or by acquisition of DHPS drug resistance genes (*sul* genes), whose products have lower affinities for sulfonamides (2, 7, 8, 13, 16–19). For many years, only two DHPS drug resistance genes, *sul1* and *sul2*, which share 57% nucleotide sequence (DNA) identity, were detected. While *sul1* is part of the 3′ conserved segment of class 1 integrons, *sul2* often appears to be associated with genes that confer resistance to streptomycin (*strA* and *strB*). Both genes have been found on plasmids and chromosomes (2, 7, 8, 13, 16–19). The *sul1* and *sul2* genes seem to be equally distributed among sulfonamide-resistant *Escherichia coli* isolates of clinical origin (6–8, 17, 18). In some studies, only 70% of sulfonamide resistance could be explained by these genes (10; unpublished data).

Recently, Perreten and Boerlin (13) described a new DHPS sulfonamide resistance gene, designated *sul3* (GenBank accession number AJ459418), which has 50.4% amino acid identity to *Salmonella enterica* plasmid pHCM1, 40.6% amino acid identity to *sul2* from *E. coli* plasmid RSF1010, and 40.9% amino acid identity to *sul1* from *E. coli* plasmid R388 (GenBank accession number X12869). This *sul3* gene was found in Swiss *E. coli* strains from pigs. For *Salmonella*, a gene named *sul3* can be found in GenBank (accession number AY047357); however, this gene seems to be a defective *sul1* gene. The objective of this work was to ascertain the presence and spread of the *sul3* gene described for the Swiss *E. coli* isolates in nontyphoid *Salmonella* strains.

The study included 512 epidemiologically unrelated German sulfonamide-resistant *Salmonella* strains from the German National *Salmonella* Reference Laboratory (NRL-Salm; Berlin, Germany) strain collection. They were isolated in 2001 from livestock (100, 87, and 75 isolates from poultry, cattle, and swine, respectively), food products (236 isolates), and feed (14 isolates) in different laboratories in the 16 German states (Länder).

Several steps were used in the study. (i) Dot blotting was used to screen the isolates for the *sul3* gene. For dot blotting, 5 μl (about 100 ng) of boiled DNA (11) was spotted onto nylon membranes (Roche Applied Sciences, Mannheim, Germany), cross-linked for 2 min, and hybridized by a nonradioactive method (Roche Applied Sciences) with a *sul3*-specific probe obtained from plasmid pVP440 (13). (ii) PCR amplification was then used to confirm the presence of *sul3* in the suspected positive isolates. PCR was carried out as described previously (4) with primers *sul3*-F (GAGCAAGATTTTTGGAATCG) and *sul3*-B (CATCTGCAGCTAACCTAGGGCTTTGGA) and the conditions described elsewhere (13). (iii) The sequence of the *sul3* gene found in one *Salmonella*-positive strain was then analyzed. First, only the PCR product obtained with the *sul3*-specific primers was sequenced, as described previously (4). Second, the 5′ flanking region was also sequenced by using internal primers specific for the *orf* and *sul3* genes (GenBank accession number AJ459418). (iv) Restriction fragment length polymorphism (RFLP) analysis of all the *sul3* PCR products was then performed as described previously (4) with 5 U of HindIII or SphI endonuclease (Amersham Biosciences, Freiburg, Germany). (v) Molecular typing of the strains was performed by plasmid analysis (9) and pulsed-field gel electrophoresis (PFGE) with the XbaI endonuclease (11) and the run conditions recommended by the Salm-Net project (12). (vi) Finally, the location of the *sul3* gene was determined by Southern blot hybridization (14) of the plasmid patterns with the *sul3*-specific probe.

Twenty-two of the 512 (4.3%) sulfonamide-resistant *Salmonella* strains (Table 1) hybridized with the *sul3*-specific probe. By PCR, all 22 strains gave amplification products of about 789 bp. The sequence of the PCR product generated by *Salmonella* strain NRL-01-02571 showed 100% identity with the sequence of the *sul3* gene found in *E. coli* deposited in GenBank (accession number AJ459418). Restriction of all *sul3* PCR products with the HindIII or the SphI endonuclease generated the same RFLP patterns generated for the control (fragments of 591 and 198 bp and fragments of 700 and 89 bp, respectively).

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TABLE 1. Features of the *Salmonella* strains carrying the *sul3* gene

NRL reference strain no.	Serotype (phage type)	German region of isolation	Origin	Resistance pattern[a]	PP	PFP	Amplification by PCR[b]		
							sul3	*sul1*	*sul2*
01-00835	Agona	Brandenburg	Turkey	AMP-CHL-STR-SPT-SUL-TET	14	X13	+	−	−
01-01887	Agona	Nordrhein-Westfalen	Turkey	AMP-CHL-KAN-NEO-STR-SPT-SUL-TET	9	X13	+	−	−
01-01055	Agona	Brandenburg	Turkey	CHL-STR-SPT-SUL	8	X14	+	−	−
01-01647	Anatum	Nordrhein-Westfalen	Turkey	CHL-STR-SPT-SUL	13	X15	+	−	−
01-00463	Brandenburg	Brandenburg	Turkey	CHL-STR-SPT-SUL	4	X11	+	−	−
01-00799	Brandenburg	Bayern	Chicken	AMP-CHL-KAN-NEO-STR-SPT-SUL-TET	6	X12	+	−	−
01-00461	Heidelberg	Brandenburg	Turkey	CHL-STR-SPT-SUL-TET	3	X1	+	−	−
01-00564	Heidelberg	Brandenburg	Turkey	CHL-STR-SPT-SUL	3	X1	+	−	−
01-00867	Heidelberg	Brandenburg	Turkey	AMP-CHL-STR-SPT-SUL	3	X1	+	−	−
01-00830	Heidelberg	Brandenburg	Turkey	AMP-CHL-STR-SPT-SUL-TET	3	X2	+	−	−
01-01645	Heidelberg	Thüringen	Turkey	CHL-KAN-NEO-STR-SPT-SUL	3	X1	+	−	−
01-01744	Heidelberg	Rheinland-Pfalz	Poultry meat	CHL-STR-SPT-SUL	3	X1	+	−	−
01-00466	Heidelberg	Brandenburg	Turkey	AMP-CHL-KAN-NEO-STR-SPT-SUL-TET	5	X4	+	−	−
01-00828	Heidelberg	Brandenburg	Turkey	CHL-STR-SPT-SUL	11	X5	+	−	−
01-00973	Heidelberg	Brandenburg	Turkey	AMP-CHL-KAN-NEO-STR-SPT-SUL-TET	7	X3	+	−	−
01-01743	Heidelberg	Rheinland-Pfalz	Turkey meat	CHL-STR-SPT-SUL	12	X1	+	−	−
01-00617	Subspecies I, rough[c]	Brandenburg	Turkey	CHL-KAN-NEO-STR-SPT-SUL	3	X1	+	−	−
01-00614	Subspecies I, rough	Brandenburg	Turkey	CHL-STR-SPT-SUL	3	X10	+	−	−
01-00055	Subspecies I, rough	Bayern	Chicken	CHL-KAN-NEO-SPT-SUL-TET	2	X8	+	−	−
01-00398	Subspecies I, rough	Bremen	Swine meat	AMP-CHL-STR-SPT-SUL-TET-TMP-SXT	10	X9	+	−	−
01-03713	Monophasic [4,5,12:i:−] (U302)	Sachsen	Swine meat	AMP-CHL-GEN-STR-SPT-SUL-TET-TMP-SXT	15	X7	+	−	+
01-02571	Typhimurium (DT104A)	Sachsen-Anhalt	Swine	AMP-CHL-FFN-STR-SPT-SUL-TET-TMP-SXT	1	X6	+	−	−

[a] The MICs of 17 antimicrobial agents were assessed by the NCCLS broth microdilution method as described elsewhere (6). AMP, ampicillin; CHL, chloramphenicol; FFN, florfenicol; GEN, gentamicin; KAN, kanamycin; NEO, neomycin; STR, streptomycin; SPT, spectinomycin; SUL, sulfamethoxazole; TET, tetracycline; TMP, trimethoprim; SXT, trimethoprim-sulfamethoxazole.

[b] PCR amplifications were done with the primers described previously for *sul1* (15) and *sul2* (1).

[c] The strains did not agglutinate with the specific sera used.

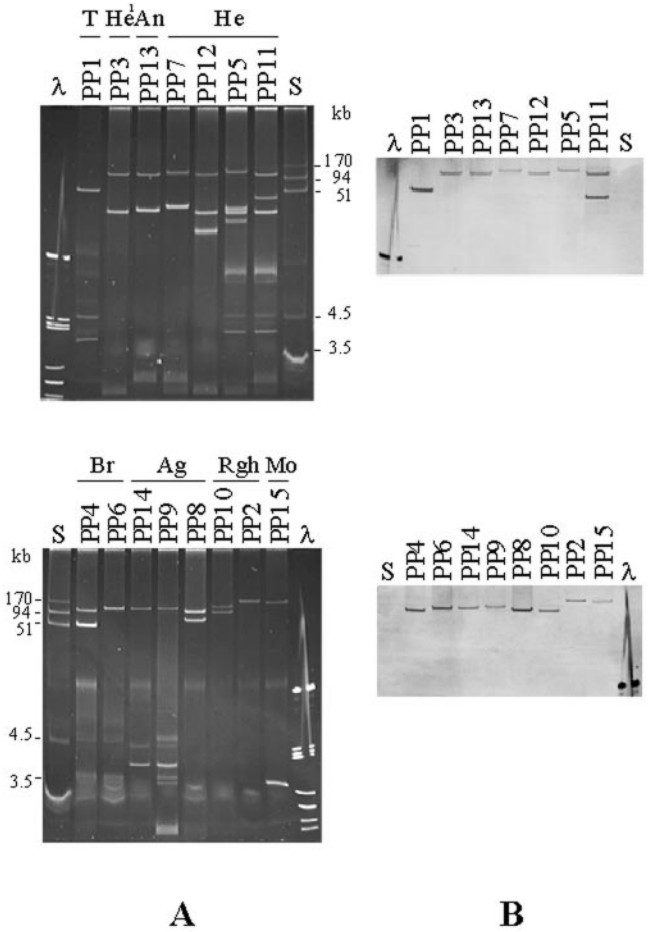

FIG. 1. Plasmid analysis of the *Salmonella* strains carrying the *sul3* gene. (A) plasmid patterns; (B) hybridization of the plasmid DNA in panel A with a *sul3*-specific probe. Lanes: λ, bacteriophage λ DNA digested with PstI; S, plasmids R27, R1, and V157 used as size standards (170 to 1.9 kb); T, *Salmonella* serotype Typhimurium; He, *Salmonella* serotype Heidelberg; An, *Salmonella* serotype Anatum; Br, *Salmonella* serotype Brandenburg; Ag, *Salmonella* serotype Agona; Rgh, rough; Mo, monophasic. [1], the same plasmid profiles were shown by rough strains.

Seventeen of the *sul3* carrier strains were from poultry livestock, one was from swine livestock, and four were from food products (two from poultry products and two from swine products) (Table 1). The poultry isolates were predominately isolated from turkeys or turkey products (at least 16 isolates). The *sul3* gene could not be detected among isolates originating from cattle or feed.

The strains carrying *sul3* belonged to different serotypes, most of them to *S. enterica* serotype Heidelberg (10 strains), and originated from 8 of the 16 German states, although most of them came from the Brandenburg region (12 strains). The strains were very heterogeneous, showing nine different antimicrobial resistance patterns (RPs), 15 plasmid profiles (PPs), and 15 XbaI PFGE patterns (PFP-X) (Table 1; Fig. 1 and 2). The predominant genomic group was represented by five *Salmonella* serotype Heidelberg strains and one rough strain, which showed PP3 and PFP-X1. Only one strain carried a second sulfonamide resistance gene (*sul2*).

The *sul3* gene was located on large plasmids of different sizes, most of which were >90 kb (Fig. 1). One strain presented two copies of the gene on different plasmids.

The widespread resistance to sulfonamides is a good example of rapid adaptive evolution due to the horizontal transfer of resistance genes among mixed bacterial populations. Even though the use of sulfonamides in human medicine has decreased, selection pressures still exist in the veterinary, agriculture, and aquaculture fields in some countries (2, 7, 8, 18). Consequently, the genetic determinants for sulfonamide resistance are still very common in gram-negative bacterial plasmids. This persistence seems to be related to the incorporation of these determinants into very efficient vehicles for their spread: *sul1* in class 1 integrons and *sul2* in small multicopy plasmids or large transmissible multiresistance plasmids (1, 2, 5, 7, 17–19). The spread of the *sul3* genes found in the Swiss *E. coli* isolates seems to be related to transposable elements (13; V. Perreten, personal communication).

The present work describes for the first time the presence of the *sul3* gene in *Salmonella* strains. We have shown that *sul3* can be found on different large plasmids and that it is not only spread among *E. coli* strains of different origins and from different countries (3, 6, 13). *Sul3* can be detected in *Salmonella* strains of different origins, serotypes, and genomic groups. This study highlights the strong potential for the wide distribution of the *sul3* resistance determinant in bacterial populations.

Nucleotide sequence accession number. The *sul3* gene found in *Salmonella* strains has been submitted to GenBank and can be found under accession number AY316203.

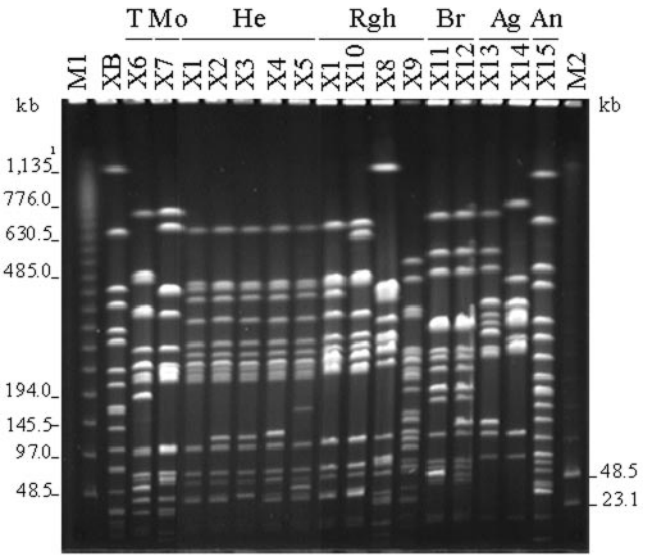

FIG. 2. XbaI PFGE patterns of the *Salmonella* strains carrying the *sul3* gene. Lanes: M1 and M2, Lambda Ladder and Low Range PFG-Markers (New England Biolabs, Schwalbach, Germany), respectively; XB, reference strain *Salmonella* serotype Braenderup H9812 (Centers for Disease Control and Prevention); T, *Salmonella* serotype Typhimurium; Mo, monophasic; He, *Salmonella* serotype Heidelberg; Rgh, rough; Br, *Salmonella* serotype Brandenburg; Ag, *Salmonella* serotype Agona; An, *Salmonella* serotype Anatum. [1], the size of the largest fragment of serotype Braenderup strain H9812.

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