

# Association of Composite IS26-*sul3* Elements with Highly Transmissible IncI1 Plasmids in Extended-Spectrum- $\beta$ -Lactamase-Producing *Escherichia coli* Clones from Humans<sup>∇</sup>

Tânia Curiao,<sup>1,3</sup> Rafael Cantón,<sup>1,2,3</sup> M. Pilar Garcillán-Barcia,<sup>4</sup> Fernando de la Cruz,<sup>4</sup>  
Fernando Baquero,<sup>1,2,3</sup> and Teresa M. Coque<sup>1,2,3\*</sup>

*Servicio de Microbiología, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain<sup>1</sup>; Unidad de Resistencia a Antibióticos y Virulencia Bacteriana Asociada al Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain<sup>2</sup>; CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain<sup>3</sup>; and Departamento de Biología Molecular e Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC), Universidad de Cantabria-CSIC-IDICAN, C. Herrera Oria s/n, 39011 Santander, Spain<sup>4</sup>*

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**The association of an IS440-*sul3* platform with Tn21 class 1 integrons carried by IncI1 plasmids encoding extended-spectrum  $\beta$ -lactamases (ESBLs; mainly SHV-12 and CTX-M-14) among worldwide *Escherichia coli* clones of phylogroups A (ST10, ST23, and ST46), B1 (ST155, ST351, and ST359), and D/B2 (ST131) is reported. An *in silico* comparative analysis of *sul3* elements available in the GenBank database shows the evolution of *sul3* platforms by hosting different transposable elements facilitating the potential genesis of IS26 composite transposons and further insertion element-mediated promoted arrangements.**

Acquired resistance to sulfonamides is due to the presence of dihydropteroate synthase (DHPS) genes located on class 1 integrons (*sul1* and *sul3*) or genetic islands bearing Tn5393 and ISCR2 (*sul2*) (14, 23, 25). Since its first description in the early 1960s, there is evidence of the spread of plasmids containing *sul1* and *sul2* among different hosts (10, 12, 13, 19, 26). The *sul3* integrons have been increasingly reported among animals and, to a lesser extent, among humans since they were identified in the mid-1990s (2, 4, 11, 23, 24, 32). However, the genetic elements linked to their spread remain scarcely explored (2, 23, 32).

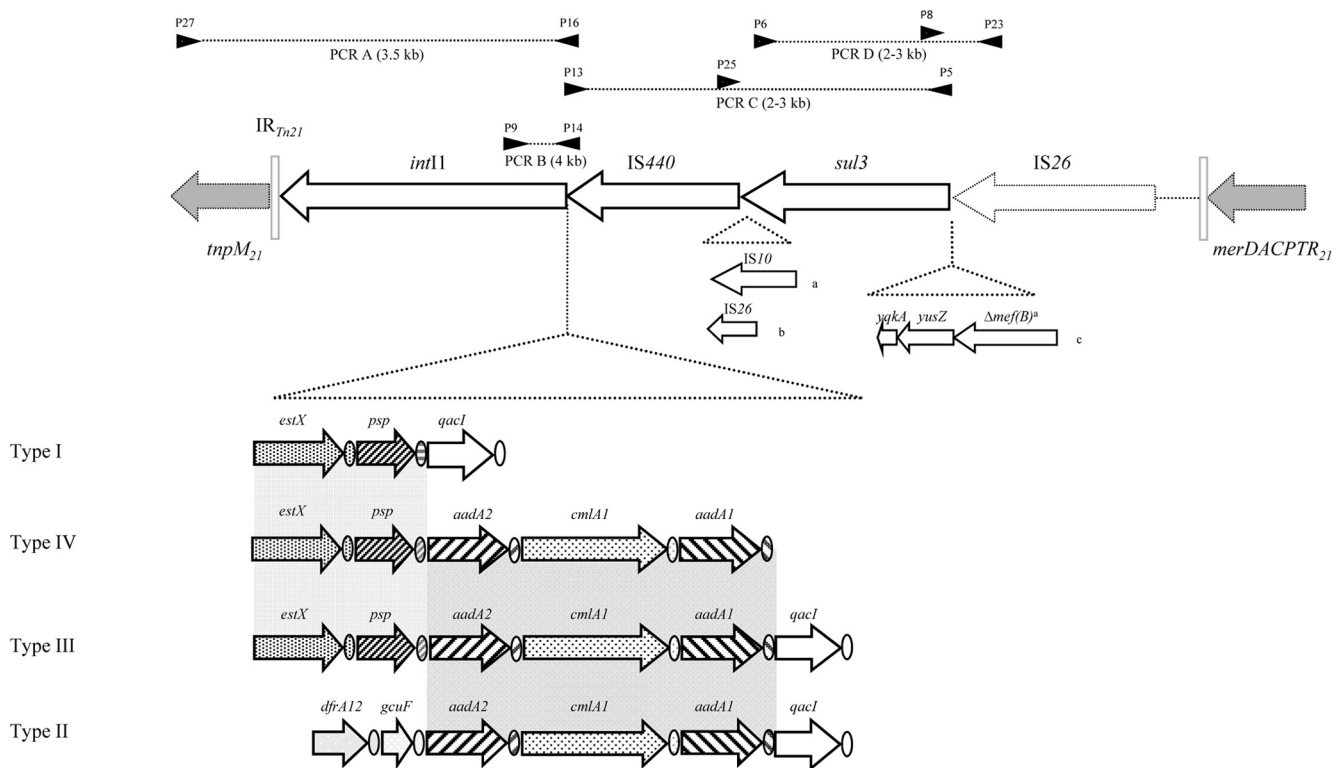
We analyzed 344 clonally unrelated *Enterobacteriaceae* isolates (249 *Escherichia coli*, 56 *Klebsiella pneumoniae*, 20 *Enterobacter cloacae*, 3 *Enterobacter aerogenes*, 7 *Klebsiella oxytoca*, 6 *Salmonella enterica* serovar Paratyphi, and 3 *Citrobacter* isolates). They included 244 extended-spectrum- $\beta$ -lactamase (ESBL) or metallo- $\beta$ -lactamase (MBL) producers obtained from hospitalized and healthy humans (1988 to 2006) and 100 non-ESBL producers (66 obtained from blood samples from inpatients and 34 obtained from feces samples from healthy volunteers without recent exposure to antibiotics or hospital environments; 1988 to 2006) (see Table 2). Species identification and susceptibility testing were performed by using the automated WIDER system (Fco. Soria Melguizo, Madrid, Spain) and standard methods (7). Clonal relatedness among *Escherichia coli* isolates was established by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) (30; <http://www.mlst.net>), and determination of phylogenetic groups by a multiplex PCR assay (6).

The *sul3* gene was detected in 6% of the strains (22 strains from 12 hospitalized patients, 8 outpatients, and 2 healthy humans; 1997 to 2006). All were ESBL/MBL-producing *E. coli* strains showing different PFGE patterns and sequence types (ST) linked to phylogroups A ( $n = 9$ ; ST10, ST23, ST46, ST156, and ST695), B1 ( $n = 6$ ; ST155, ST351, and ST359), and D/B2, ( $n = 7$ ; ST131, ST350, ST624, and ST648). The most common ESBLs/MBLs were SHV-12 ( $n = 13$ ) and CTX-M-14 ( $n = 8$ ), followed by CTX-M-15 ( $n = 2$ ), CTX-M-1, CTX-M-9, VIM-1, and TEM-24 ( $n = 1$  each) (see Table 2). Four strains produced two ESBL/MBL enzymes (CTX-M-14, CTX-M-15, or VIM-1 plus SHV-12). The *sul3* strains often carried other *sul* genes (*sul1*, *sul2*, and *sul3*,  $n = 16/22$ ; *sul1* and *sul3*,  $n = 2$ ; *sul2* and *sul3*,  $n = 1$ ; *sul3* only,  $n = 3$ ) and expressed resistance to sulfonamides (86%), streptomycin (86%), trimethoprim (77%), tetracycline (77%), and chloramphenicol (64%). The low prevalence of the *sul3* gene is similar to that reported by other studies (2, 4, 15), in contrast to that reported for the *sul1* or *sul2* gene (14, 19, 26). The association of *sul3* with ESBL *E. coli* producers with zoonotic potential (phylogroup B2 *E. coli* O25:H4-ST131 and phylogroup D *E. coli* O25a-ST648, -ST69, and -ST393) (8, 31) highlights the role of these frequent clones in the evolution of antibiotic resistance to sulfonamides and beta-lactams in areas of common exposure to these antibiotics, such as farms or hospitals.

Characterization of *sul3* class 1 integrons and linkage to Tn21 derivatives were accomplished by analyzing the presence of *intI1*, *sul3*, *qacI*, *tmpM<sub>21</sub>*, and *mer<sub>21</sub>* by PCR/hybridization and further PCR mapping (Fig. 1 and Table 1). The diversity of *sul3* platforms was established by comparison of restriction fragment length polymorphism (RFLP) patterns of HindIII-, EcoRI-, or PstI-digested amplicons, primer walking sequencing of representatives types, and further analysis of all *sul3* elements available in the GenBank database. We detected four *sul3* integron arrangements arbitrarily designated types I<sub>S3</sub> to

\* Corresponding author. Mailing address: Servicio de Microbiología, Hospital Universitario Ramón y Cajal, Carretera de Colmenar, km 9.1., 28034 Madrid, Spain. Phone: 34-913368330. Fax: 34-913368809. E-mail: mcoque.hrc@salud.madrid.org.

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Integron type	Subtype	Host	Year	N° of isolates	PCR A (tnp2I)	PCR B	PCR C	PCR D <sup>a</sup>	merA	GenBank acc. no.
I	-	<i>E. coli</i>	2000-01	2	-	+	+	-	-	HQ875011
II	-	<i>E. coli</i>	2000-02	2	-	+	+	-	-	HQ875012
	a	<i>E. coli</i>	2002	1	+	+	++	-	+	HQ875013
III	-	<i>E. coli</i>	2002-06	8	(+) 3	+	+	(++) 1	(+) 4	-
	b	<i>E. coli</i>	2002	1 <sup>b</sup>	-	+	++	++	-	HQ875016
	c	<i>E. coli</i>	1997-2004	6	(+) 5	+	+	+	(+) 4	HQ875014; HQ875015
IV <sup>c</sup>	-	<i>E. coli</i>	2002	1	+	-	-	-	-	HQ875017

FIG. 1. Schematic representation of *sul3* genetic elements characterized by PCR mapping based on the Tn402 and Tn21 sequences. (Top) The locations of the primers used for the PCR mapping assay are represented with black arrowheads. P13 and P14 hybridize in the *qacI* gene. (Middle) The *attC* sites or 59-base elements were represented by circles patterned as corresponding gene cassettes. (Bottom) Preliminary screening of *sul3* elements related to Tn402 and Tn21 was performed as shown in the table. ++, amplicon of larger size; (+), variable amplification results, with the number of positive results indicated; <sup>a</sup>, *mefB* was complete in only two isolates, and in most cases, the gene was truncated at different points by IS26 (GenBank accession no. HQ875016, HQ875014, and HQ875015); <sup>b</sup>, this isolate harbors an integron with insertions b and c; <sup>c</sup>, integron amplification was reached using primers P9 and P5. *tnp21* was found upstream of the integrases of different *sul3* integron types ( $n = 10$ ), and a copy of IS26 was detected downstream of the *sul3* gene ( $n = 8$ ). Coexistence of both of the boundaries was detected for 7 isolates. The presence of the *mer* operon of Tn21 was inferred by *merA*.

IV<sub>S3</sub> (Table 2; Fig. 1). The predominant one was type III<sub>S3</sub>, *intI1-estX-*psp*-aadA2-cmlA1-aadA1-qacI-IS440-sul3*, present in 15 ESBL/MBL *E. coli* strains in our collection since 1997 and also globally distributed among *Enterobacteriaceae* of different origins (2, 3, 16, 17, 28). Two integrons similar to type III<sub>S3</sub> were *intI1-estX-*psp*-qacI-IS440-sul3* (type I<sub>S3</sub>) and *intI1-estX-*psp*-aadA2/aadA1a-qacI-IS440-sul3* (from farm animals in

Switzerland; GenBank accession no. FM242710) (Fig. 2). Differences in *attC* carried by the *psp* and *aadA2* gene cassettes of different integrons suggest arrangements. Type II<sub>S3</sub>, *intI1-dfrA12-gcuF-aadA2-cmlA1-aadA1-qacI-IS440-sul3*, has also been detected in humans, animals, and foods in Europe and Asia since 2003. Finally, a few *sul3* platforms lacking *qacI* included *intI1-estX-*psp*-aadA2-cmlA1-aadA1-IS440-sul3* (type

TABLE 1. Oligonucleotides used in this study<sup>b</sup>

Primer no.	Primer	Sequence (5'-3')	GenBank accession no.	Nucleotide positions	Source/reference(s)
1	<i>sul1F</i>	CGGCGTGGGCTACCTGAACG	EU622038	3508–3528	15
2	<i>sul1R</i>	GCCGATCGCGTGAAGTTCCG	EU622038	3921–3940	15
3	<i>sul2F</i>	GCGCTCAAGGCAGATGGCATT	M36657	534–555	15
4	<i>sul2R</i>	GCGTTTGATACCGGCACCCGT	M36657	819–798	15
5	<i>sul3F</i>	GAGCAAGATTTTTGGAATCGT	AJ459418	2980–3001	23
6	<i>sul3R</i>	CTAACCTAGGGCTTTGGATA	AJ459418	3770–3750	This study
7	<i>sul3F2</i>	TATCCAAAGCCCTAGGTTAG	AJ459418	3750–3770	This study
8	<i>sul3R2</i>	GAACTACGACTGGTTTC	AJ459418	2797–2780	This study
9	<i>Int11F</i>	GGGTCAAGGATCTGGATTTTCG	AF071413	4775–4755	21
10	<i>Int11R</i>	ACATGCGTGTAAATCATCGTCC	AF071413	4333–4312	21
11	5'CS	GGCATCCAAGCAGCAAG	AF174129	1236–1252	21
12	3'CS	AAGCAGACTTGACCTGAT	AF174129	2813–2830	21
13	<i>qac1F</i>	ACTGGCTCTTTCTGGCTATT	EF051039	5064–5084	This study
14	<i>qac1R</i>	TAACGATAAGTCCCATGCCA	EF051039	5343–5323	This study
15	<i>cmlA1F</i>	CACCTCAAGAAGCGACACA	EF051039	2621–2641	This study
16	<i>cmlA1R</i>	TTCCGATGCTTCCTAGCAGT	U12338	8020–8000	This study
17	<i>cmlA1FR</i>	ACTGCTAGGAAGCATCGGAA	EF051039	3823–3803	This study
18	<i>aadA2R</i>	TGACTTGATGATCTCGCC	AF174129	2692–2709	21
19	<i>estX</i>	TTCTTATGTGCATGGTT	EF051039	794–813	This study
20	<i>dfrA12F</i>	TTACGTCCAACGTTAGCAC	EF051037	1088–1107	This study
21	<i>aadA1R</i>	ATTGCGCTGCCATTCTCCA	EF051037	4179–4160	This study
22	<i>psp R</i>	ATCAGGGTGCCAGACAAGA	EF051039	1189–1170	This study
23	IS26F	AGCGGTAAATCGTGGAGTGA	AF205943	324–344	21
24	IS26R	AGGCCGGCATTTCAGCGTG	AF205943	979–960	21
25	IS440R	TGCGGGTACTTACTCCTTG	FJ587511	6415–6396	This study
26	<i>Orf1R</i>	GCAATGCCATTAGATTCATC	FJ196385	10347–10327	This study
27	TnpM Fw	CCGTGGTGGTGCATAGCAT	AF071413	4020–4002	21
28	<i>merA1</i>	ACCATCGGGCGCACCTGCGT	AF071413	17597–17578	21
29	<i>merA5</i>	ACCATCGTCAGGTAGGGGAACAA	AF071413	16360–16382	21
30	<i>copAF</i>	ATGCGCCATAAGGCATTCA	NC_0050144	215–234	30
31	repAR	AGTCGCTTCAGATGGTCAT	NC_005014	1427–1408	30
32	mobP12F	GCAAAAAGATGACACTGAYCCYGT	NC_005014	67717–67743	1, 30
33	mobP12R	AGCGATGTGGATGTGAAGTTAT	NC_002122	31165–31120	1, 30
34	<i>ardAF</i>	ATGCTGTGTGGTGCACCTGC	AP005147	61469–61811	PubMLST <sup>a</sup>
35	<i>ardAR</i>	TCACCGACGGAACACATGACC	AP005147	61926–61906	PubMLST
36	<i>trbAF</i>	GCGGTTATCGGGGCTACTA	AP005147	74976–74959	This study
37	<i>pndAF</i>	GAATTCGTTGTCTGTAGCA	AP005147	73503–73521	This study
38	<i>sogSF</i>	TTCCTGGGCGTAGACAATACT	AP005147	93088–93108	PubMLST
39	<i>sogSR</i>	AACAGTGATATGCCGTCGC	AP005147	93378–93360	PubMLST
40	<i>pilVF</i>	CCATATGACCATCCAGTGCG	AP005147	114765–114784	PubMLST
41	<i>pilVR</i>	AACCACTATCTCGCCAGCAG	AP005147	115080–115061	PubMLST

<sup>a</sup> [http://pubmlst.org/perl/bigdb/bigdb.pl?db=pubmlst\\_plasmid\\_seqdef](http://pubmlst.org/perl/bigdb/bigdb.pl?db=pubmlst_plasmid_seqdef).<sup>b</sup> The primer sequences used in primer walking assays are available upon request.

IV<sub>S3</sub>; GenBank accession no. HQ875017), *int11-dfrA12-gcuF-aadA2-cmlA1-aadA1-IS440-sul3* (27), and partial sequences *aadA1-IS440-sul3-orf1-mefB* and *aadA3-IS440-sul3-orf1-mefB* (GenBank accession no. FJ196384 and FJ196388, respectively). The *sul3* integrons obtained from *E. coli* and *Salmonella enterica* isolates from humans and farm animals were associated with Tn21 platforms often interrupted by IS26 at different positions (Fig. 1 and 2). Analysis of sequences revealed that a genetic platform, IS440-*sul3-orf1-mefB*, is specifically located beyond the *attC* sites of the *qacI*, *aadA1*, and *aadA3* gene cassettes of integrons (Fig. 2). The lack of identifiable boundaries for IS440, a putative IS256 family member, reflects either a single insertion downstream of the last gene cassette (most probably *qacI*) of an ancestral integron, followed by further excisions and insertions of different gene cassettes or independent insertions at the end of different *attC*

sites. The existence of identical integron arrangements lacking and containing IS26 and IS10 surrounded by direct repeats of the original target sequence indicates that the IS440-*sul3* platform, once acquired, has been the target of further lateral transfer events. Moreover, complete or partial copies of IS26 at the boundaries of different integrons led to the genesis of putative IS26 composite transposons (Fig. 1 and 2). The lack of target site duplication at the sides of these IS26 copies might indicate the transference of either IS26 composite elements by homologous recombination among diverse plasmids or larger platforms in which they were located.

Plasmids carrying *sul3* belonged to a diversity of IncI1, IncY, and IncB/O groups (55 to 220 kb), mostly conjugative ones (86%). They were identified by PCR typing methods targeting replication and conjugation sequences of plasmids of *Enterobacteriaceae* (1, 5) and further hybridization of S1 nuclease-

TABLE 2. Epidemiological data of isolates producing *sul3* integrons in Hospital Ramón y Cajal<sup>a</sup>

Integron type (no. of isolates)	<i>sul3</i> plasmid (kb) <sup>b</sup>	Inc. group(s) (no. of isolates)	RFLP type (no. of isolates) <sup>c</sup>	pMLST InclI <sup>d</sup>			ESBL(s) (no. of isolates)	<i>E. coli</i> MLST result(s)	Yr	Origin (no. of isolates)	Antibiotic resistance <sup>e</sup>	<i>sul</i> gene(s) (no. of isolates) <sup>f</sup>
				<i>andA</i>	<i>trbA-pmdA</i>	<i>sogS</i>						
I (2)	<u>100</u>	II, B/O	A (2)	4	3	DLV 4	1	CTX-M-14	ST57, ST350	2000–2001	Urine/P	Sm, Na, Su, W, (Te), Ch, Km <i>sul1</i> (1)
II (3)	125	Y	G	4	16	9	2	SHV-12	ST48/ST10	2000	Blood/P	Sm, (Sp), (Cip), (Na), Su, W, Ch, (Ap), (Te) <i>sul2</i>
	125, 55 100	B/O and NT <sup>f</sup> II, B/O	ND F	4	4	9	2	SHV-12 CTX-M-1	ST131	2000 2002	Feces/HV Urine/P	
III (15)	<u>100</u>	II (6)	B (6)	4	3	DLV 4	1	SHV-12, CTX-M-14 (2), CTX-M-15 (1), VIM-1 (1)	ST359, ST155, DLV ST155, ST156, ST46; ND	1997–2006	Urine (2)/P, blood/P, feces/HV, gangrene/P	(Sm), (Sp), (Na), (Su), (W), (Te), (Ch), (Nt), (Km), (Gm), (Tb), (Ak) <i>sul1, sul2</i>
	150 100 100 100 100 <u>50</u>	FIB+B/O/ II+B/O/ II, B/O II, B/O II, K, FIB, F	D C1 C2 ND	4	3	DLV 4	1	SHV-12	ST695	2002	Urine/P	<i>sulL, sul2</i>
	<u>70</u> <u>90</u> <u>105</u>	Y, FIA, F K, B/O, F A/C2, F		4	3	DLV 4	1	SHV-12 CTX-M-14	ST10 ST359 ST167	2002 2002	Urine/P Urine/P	<i>sul1, sul2</i> <i>sul1</i> <i>sul2, sul2</i>
	150 220	FIA, (no F) II, N, B/O		4	13	2	1	SHV-12 CTX-M-14	ST167	2001–2002	Urine (3)/P, cutaneous/P, genital/P, epidemic surveillance/P	<i>sul1, sul2</i> <i>sul1, sul2</i> <i>sul1, sul2</i>
IV (1)	<u>100</u>	II	E	1	4	1	2	SHV-12	ST23	2002	Urine/P	Sm, Sp, Na, Su, W, Ch, Te <i>sulL, sul2</i>

<sup>a</sup> *sul3* was detected among a collection of strains which included (i) 244 ESBL or MBL producers [TEM (-4, -12, -24, -27, and -52), SHV (-2, -2a, -5, -12, and -13), CTX-M (-1, -3, -9, -10, -14, -15, and -32), VIM-1, and OXA-30], representatives of ESBL clones recovered in Hospital Ramón y Cajal from 1988 to 2006, a collection partially explored in other studies with other purposes (18, 19, 21, 30) (1988 to 2006) and (ii) 66 non-ESBL isolates from blood samples obtained from different inpatients and 34 fecal isolates obtained from healthy volunteers living in the Madrid area (18). Abbreviations: Sm, streptomycin; Sp, spectinomycin; Na, nalidixic acid; Cip, ciprofloxacin; Te, tetracycline; Sul, sulfonamide; W, trimethoprim; Ch, chloramphenicol; Ak, amikacin; Km, kanamycin; Gm, gentamicin; Nt, netilmicin; Tb, tobramycin; Ap, apramycin; DLV, double-locus variant; NT, not typeable; ND, not done; P, hospitalized patient; HV, healthy volunteer. Names in boldface indicate either an ESBL encoded by *bla* genes or a *sul2* gene located on plasmids carrying *sul3*.

<sup>b</sup> Plasmids transferred by conjugation to *E. coli* K-12 BM21 (rifampin and nalidixic acid resistant, Lac<sup>+</sup>, and plasmid free) are underlined (18).

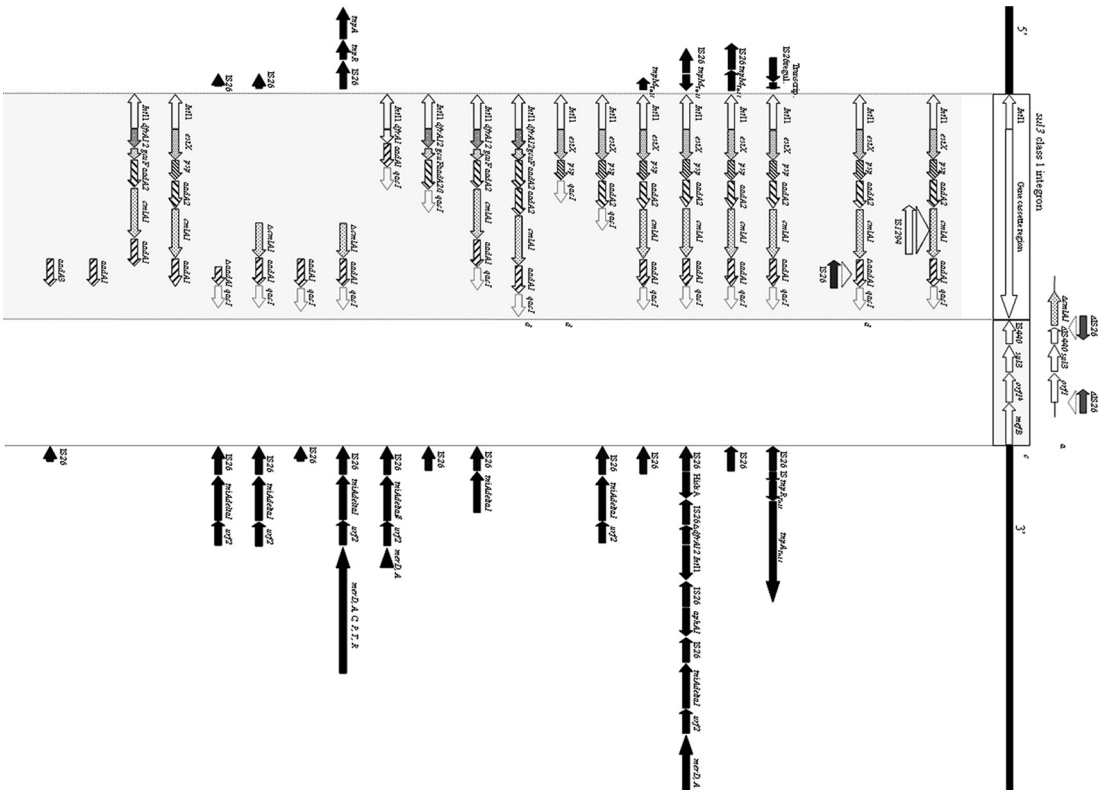
<sup>c</sup> Detailed analysis of 13 InclI-like transferable plasmids revealed 8 RFLP patterns, designated with capital letters (A to G). Plasmids from six isolates were not studied further since they were cotransferred with other plasmids carrying different ESBL genes.

<sup>d</sup> Diversity of five genes corresponding to the backbone of InclI plasmids (*epfY* = replicase, see the text; *ardA* = gene encoding a type I restriction-modification enzyme; *trbA* = gene involved in maintenance and plasmid transfer; *pmdA* = gene involved in plasmid transfer; *sogS* = gene coding for a DNA primase; *pilV* = gene associated with type IV plus biogenesis) following the allele designation given and used in the MLST scheme proposed by Alessandra Carattoli ([http://pubmlst.org/perl/bigsdh/pi?db=pubmlst\\_plasmid\\_seqdef](http://pubmlst.org/perl/bigsdh/pi?db=pubmlst_plasmid_seqdef)). Numbers represent different alleles for each gene.

<sup>e</sup> Susceptibility to non-beta-lactam antibiotics was determined by using the standard disk diffusion method (7). Antibiotics in parentheses indicate that resistance was not present in all isolates. Cotransference with *sul3* is indicated by underlining.

<sup>f</sup> Strains containing two copies of a given *sul3* integron carried by different plasmids.

<sup>g</sup> Underlined genes were cotransferred with *sul3*.



Genetic context	Hosts	Origin	Regions	Year	GenBank acc. nos.	References
IS26f	<i>E. coli</i>	Fecal (healthy humans)	Spain	2004	EU704128	27
UNK f	<i>E. coli</i>	Turkey meat	Tunisia	2008	FJ160769	28
pCVM19633_100 (InclI)	<i>S. enterica</i>	-	USA	2008	NC_011092	Upp
UNK f	<i>S. enterica</i>	Human, Swine	Taiwan	2000-01	E0334941	16
pSC138 (InclI/F)	<i>S. enterica</i>	Human	Taiwan	2002	AY509004	Upp
pOU75197 (InclI/FIB)	<i>S. enterica</i>	-	Taiwan	2007	EU219534	-
UNK f	<i>E. coli</i>	Swine	UK	1999	F1196385	9
TK21	<i>S. enterica</i>	Human, Swine, Poultry, Enter.	Spain, Portugal	2001-02, 2000-04	HQ875014, EF031039	This study
UNK f	<i>E. coli</i>	Farm animals	Switzerland	2009	FM242710	Upp
UNK f	<i>E. coli</i>	Human	Spain	2000-01	HQ875011	This study
UNK f	<i>E. coli</i>	Mixed meat	Norway	2009	EF113389	-
pVIZ402	<i>E. coli</i>	Farm animals	Switzerland	2009	FM244708	Upp
TK21 pBC355	<i>E. coli</i>	Farm animals	Spain	2000-02	HQ875013	This study, 27
pRYC302 f, IS26f	<i>S. enterica</i>	Food, Human, Swine	Portugal	2000-04	EF500107	2
IS26f	<i>S. enterica</i>	Pork products	Portugal	2002-03	EF051038	2
TK21	<i>E. coli</i>	Farm animals	Switzerland	2009	FM244709	Upp
pBC54	<i>E. coli</i>	Farm animals	Switzerland	2009	FM244713	Upp
TK21 pVP2820	<i>E. coli</i>	Farm animals	Switzerland	2009	FM244713	Upp
UNK f	<i>E. coli</i>	Swine	UK	1999	F1196386	9
pP328	<i>E. coli</i>	Swine	UK	1999	F1196386	9
IS26	<i>E. coli</i>	Farm animals	Switzerland	2009	FM244710	Upp
pVP1740	<i>E. coli</i>	Farm animals	Switzerland	2009	FM244712	Upp
IS26	<i>E. coli</i>	Farm animals	Switzerland	2009	FM244712	Upp
pVP1750	<i>E. coli</i>	Farm animals	Switzerland	2009	FM244712	Upp
UNK	<i>E. coli</i>	Human	Spain	2002	HQ875017	This study
pRYC305	<i>E. coli</i>	Fecal (healthy humans)	Spain	2004	-	27
IS26f	<i>E. coli</i>	Fecal (healthy humans)	Spain	2004	-	27
UNK	<i>E. coli</i>	Swine	UK	1999	F1196384	9
pP126	<i>E. coli</i>	Swine	UK	1999	F1196384	9
UNK f	<i>E. coli</i>	Swine	UK	1999	F1196388	9

FIG. 2. Diversity of the *sul3* integrons based on published studies and sequences deposited in the GenBank database. *In silico* comparative analysis was made using BLAST and CLUSTAL softwares available at the BLAST (<http://www.ncbi.nlm.nih.gov/blast/>) and ClustalW2 (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>) websites. The *sul3* gene is associated with class 1 integrons that differ from class 1 *sul1* integrons in the 3' region, which comprise the gene cassette initially annotated *qacH* but called *qacI* afterward in order to distinguish it from the unrelated *qacH* gene of *Staphylococcus aureus* (GenBank accession no. AF205943) (20, 22). <sup>a</sup>, the first described *sul3* platform recovered from an *E. coli* swine isolate in Switzerland in 2002 (GenBank accession no. AJ459418); <sup>b</sup>, *orf1* is also annotated as *ylkA-ylkZ* or *orf1-orfB* (GenBank accession no. AY509004, EU219534, FM244708, FM244709, FM244710, FM244712, and FM244713); <sup>c</sup>, insertions of IS10 or IS26 within IS440-*sul3* were found in some isolates (GenBank accession no. F1587511, HQ875013, and HQ875016) (Fig. 1); <sup>d</sup>, the *orf1-mefB* sequence was absent or not determined; <sup>e</sup>, the presence of sequences upstream of *intI1* or downstream of *intC* was not determined or was absent; <sup>f</sup>, *mefB* is absent or truncated. *aadA2* corresponding to FM242710 is a hybrid of *aadA1a* and *aadA2* containing *intC*<sub>*aadA1a*</sub>. It is annotated *aadA2* in the GenBank database.

digested genomic DNA from *E. coli* transconjugants or wild-type strains with appropriate probes (30). Characterization of IncI1 plasmids also required RFLP profile comparison and sequencing of the whole replication region, relaxase (*nikB*) (1, 30), and genes included in the plasmid MLST scheme ([http://pubmlst.org/perl/bigsgdb/bigsgdb.pl?db=pubmlst\\_plasmid\\_seqdef](http://pubmlst.org/perl/bigsgdb/bigsgdb.pl?db=pubmlst_plasmid_seqdef)). Type III<sub>S3</sub> was identified on *bla*<sub>SHV-12</sub>-IncI1 plasmids designated B, C1, C2, and D (100 to 150 kb). Genes linked to replication (*cop*, *repY*, and *repA*), transfer (*nikB*, *trbA-pndA*, *sogS*, and *pilV*), or restriction-modification systems (*ardA*) of plasmids B and D were identical to those carried by pCVM29188\_101 from *Salmonella enterica*, an IncI1 plasmid carrying *sul3* and *bla*<sub>CMY-2</sub> (GenBank accession no. CP001121). Plasmid B was identified from 1997 to 2006 in ESBL strains from different continents (data not shown). Plasmids C1 and C2 were considered IncI1-mosaic plasmids, as *repA* carried by plasmid C1 was similar to that carried by IncK plasmid R387, and plasmid C2 harbors *repA* and *sogS* alleles that are different from those harbored by pCVM29188\_101. The *repY* and *nikB* genes carried by plasmid E were identical to those carried by IncI1 pSL476\_91 and pRYC106 (GenBank accession no. NC\_011081 and GQ892053, respectively). The type I<sub>S3</sub> integron was located on IncI1 plasmids similar to those of types B and D, while the type II<sub>S3</sub> integron was linked to nontransferable IncY, IncI1/I<sub>γ</sub>, or IncB/O plasmids.

In summary, this work describes the recent spread of the IS440-*sul3* platform, facilitated by its location on highly IncI1-transferable plasmids and by IS26-promoted rearrangements among multidrug resistance transposons and/or plasmids harbored by frequent *E. coli* clones. Coselection exerted by other antibiotics and/or biocides to which sulfonamide-resistant strains are also resistant, and the low fitness cost of plasmids in which *sul* genes are located, might be responsible for the spread and persistence of *sul3*, as suggested for other genes encoding trimethoprim-sulfonamide resistance (9, 29). The concurrent emergence and dissemination of the DHPS *sul3* and ESBL genes among human *Enterobacteriaceae* since the early 1990s suggest the recent recruitment of adaptive mechanisms in different environments (animals and humans exposed to beta-lactams and sulfonamides) by the same predominant genetic platforms.

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#### REFERENCES

- Alvarado, A., M. P. Garcillán-Barcia, and F. de la Cruz. 2008. A PCR-based method for classification of conjugative plasmids based on relaxase sequences, p. 134. In Proceedings of the International Plasmid Biology Conference. International Plasmid Biology Society, Gdansk, Poland.
- 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.
- Bischoff, K. M., D. G. White, M. E. Hume, T. L. Poole, and D. J. Nisbet. 2005. The chloramphenicol resistance gene *cmlA* is disseminated on transferable plasmids that confer multiple-drug resistance in swine *Escherichia coli*. *FEMS Microbiol. Lett.* **243**:285–291.
- Byrne-Bailey, K. G., et al. 2009. Prevalence of sulfonamide resistance genes in bacterial isolates from manured agricultural soils and pig slurry in the United Kingdom. *Antimicrob. Agents Chemother.* **53**:696–702.
- Carattoli, A., et al. 2005. Identification of plasmids by PCR-based replicon typing. *J. Microbiol. Methods* **63**:219–228.
- Clermont, O., S. Bonacorsi, and E. Bingen. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl. Environ. Microbiol.* **66**:4555–4558.
- Clinical and Laboratory Standards Institute. 2009. Performance standards for antimicrobial susceptibility testing; 19th informational supplement. CLSI document M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.
- Cortés, P., et al. 2010. Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. *Appl. Environ. Microbiol.* **76**:2799–2805.
- Enne, V. I., D. M. Livermore, P. Stephens, and L. M. Hall. 2001. Persistence of sulphamamide resistance in *Escherichia coli* in the UK despite national prescribing restriction. *Lancet* **357**:1325–1328.
- Fricke, W. F., et al. 2009. Comparative genomics of the IncA/C multidrug resistance plasmid family. *J. Bacteriol.* **191**:4750–4757.
- Guerra, B., E. Junker, and R. Helmuth. 2004. Incidence of the recently described sulfonamide resistance gene *sul3* among German *Salmonella enterica* strains isolated from livestock and food. *Antimicrob. Agents Chemother.* **48**:2712–2715.
- Heuer, H., C. Kopmann, C. T. Binh, E. M. Top, and K. Smalla. 2009. Spreading antibiotic resistance through spread manure: characteristics of a novel plasmid type with low % G+C content. *Environ. Microbiol.* **11**:937–949.
- Heuer, H., and K. Smalla. 2007. Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. *Environ. Microbiol.* **9**:657–666.
- Huovinen, P., L. Sundström, G. Swedberg, and O. Sköld. 1995. Trimethoprim and sulfonamide resistance. *Antimicrob. Agents Chemother.* **39**:279–289.
- Kern, M. B., T. Klemmensen, N. Frimodt-Moller, and F. Espersen. 2002. Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of *sul* genes conferring sulphamamide resistance. *J. Antimicrob. Chemother.* **50**:513–516.
- Lee, M. F., Y. H. Chen, and C. F. Peng. 2009. Molecular characterisation of class 1 integrons in *Salmonella enterica* serovar Choleraesuis isolates from southern Taiwan. *Int. J. Antimicrob. Agents* **33**:216–222.
- Liu, J., P. Keelan, P. M. Bennett, and V. I. Enne. 2009. Characterization of a novel macrolide efflux gene, *mef(B)*, found linked to *sul3* in porcine *Escherichia coli*. *J. Antimicrob. Chemother.* **63**:423–426.
- Machado, E., et al. 2005. Integron content of extended-spectrum-beta-lactamase-producing *Escherichia coli* strains over 12 years in a single hospital in Madrid, Spain. *Antimicrob. Agents Chemother.* **49**:1823–1829.
- Machado, E., et al. 2007. Preservation of integron types among *Enterobacteriaceae* producing extended-spectrum beta-lactamases in a Spanish hospital over a 15-year period (1988 to 2003). *Antimicrob. Agents Chemother.* **51**:2201–2204.
- Naas, T., Y. Mikami, T. Imai, L. Poirel, and P. Nordmann. 2001. Characterization of In53, a class 1 plasmid- and composite transposon-located integron of *Escherichia coli* which carries an unusual array of gene cassettes. *J. Bacteriol.* **183**:235–249.
- Novais, A., et al. 2006. Dissemination and persistence of *bla*<sub>CTX-M-9</sub> are linked to class 1 integrons containing CR1 associated with defective transposon derivatives from Tn402 located in early antibiotic resistance plasmids of IncHI2, IncP1-alpha, and IncFI groups. *Antimicrob. Agents Chemother.* **50**:2741–2750.
- Partridge, S. R., G. Tsafnat, E. Coiera, and J. R. Iredell. 2009. Gene cassettes and cassette arrays in mobile resistance integrons. *FEMS Microbiol. Rev.* **33**:757–784.
- Perreten, V., and P. Boerlin. 2003. A new sulfonamide resistance gene (*sul3*) in *Escherichia coli* is widespread in the pig population of Switzerland. *Antimicrob. Agents Chemother.* **47**:1169–1172.
- Phuong Hoa, P. T., L. Nonaka, P. Hung Viet, and S. Suzuki. 2008. Detection of the *sul1*, *sul2*, and *sul3* genes in sulfonamide-resistant bacteria from wastewater and shrimp ponds of north Vietnam. *Sci. Total Environ.* **405**:377–384.
- Rådström, P., and G. Swedberg. 1988. RSF1010 and a conjugative plasmid contain *sulIII*, one of two known genes for plasmid-borne sulfonamide resis-

- tance dihydropteroate synthase. *Antimicrob. Agents Chemother.* **32**:1684–1692.
26. **Rådström, P., G. Swedberg, and O. Sköld.** 1991. Genetic analyses of sulfonamide resistance and its dissemination in gram-negative bacteria illustrate new aspects of R plasmid evolution. *Antimicrob. Agents Chemother.* **35**:1840–1848.
27. **Sáenz, Y., et al.** 2010. Class 1 integrons lacking *qacEDelta1* and *sul1* genes in *Escherichia coli* isolates of food, animal and human origins. *Vet. Microbiol.* **144**:493–497.
28. **Soufi, L., et al.** 2009. Prevalence and diversity of integrons and associated resistance genes in *Escherichia coli* isolates from poultry meat in Tunisia. *Foodborne Pathog. Dis.* **6**:1067–1073.
29. **Sundqvist, M., et al.** 2010. Little evidence for reversibility of trimethoprim resistance after a drastic reduction in trimethoprim use. *J. Antimicrob. Chemother.* **65**:350–360.
30. **Valverde, A., et al.** 2009. Spread of *bla*(CTX-M-14) is driven mainly by IncK plasmids disseminated among *Escherichia coli* phylogroups A, B1, and D in Spain. *Antimicrob. Agents Chemother.* **53**:5204–5212.
31. **Woodford, N., J. Turton, and D. M. Livermore.** 2011. Multi-resistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol. Rev.* **344**:3964–4100.
32. **Wu, S., A. Dalsgaard, A. M. Hammerum, L. J. Porsbo, and L. B. Jensen.** 2010. Prevalence and characterization of plasmids carrying sulfonamide resistance genes among *Escherichia coli* from pigs, pig carcasses and human. *Acta Vet. Scand.* **52**:47–54.