Global Emergence of Trimethoprim/Sulfamethoxazole Resistance in *Stenotrophomonas maltophilia* Mediated by Acquisition of *sul* Genes

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Trimethoprim/sulfamethoxazole (TMP/SMX) resistance remains a serious threat in the treatment of Stenotrophomonas maltophilia infections. We analyzed an international collection of 55 S. maltophilia TMP/SMX-sensitive (S) (n = 30) and -resistant (R) (n = 25) strains for integrons; sul1, sul2 and dhfr genes; and insertion element common region (ISCR) elements. sul1, as part of a class 1 integron, was detected in 17 of 25 TMP/SMX-R. Nine TMP/SMX-R strains carried *sul*2; 7 were on large plasmids. Five TMP/SMX-R isolates were positive for ISCR2, and 4 were linked to sul2; 2 others possessed ISCR3. Two ISCR2s were adjacent to floR. Six TMP/SMX-S isolates harbored novel ISCR elements, ISCR9 and ISCR10. Linkage of ISCR3, ISCR9, and ISCR10 to sul2 and dhfr genes was not demonstrated. The data from this study indicate that class 1 integrons and ISCR elements linked to sul2 genes can mediate TMP/SMX resistance in S. maltophilia and are geographically widespread, findings that reinforce the need for ongoing resistance surveillance.

Nosocomial Stenotrophomonas maltophilia are intrinsically resistant to a plethora of antimicrobial agents that severely limit commonly used empiric standard antimicrobial therapies. S. maltophilia is resistant to many β -lactams, β -lactamase inhibitors, and aminoglycosides (1,2). A recent survey of SENTRY (www.jmilabs.com) Antimicrobial Surveillance Program isolates indicated that the newer fluoroquinolones demonstrated good efficacy; the most active were levofloxacin (6.5% resistance) and gatifloxacin (14.1%) (3). Furthermore, the resistance to the polymyxins (20%–32%) is higher than observed in *Pseudomonas aeruginosa* (3,4). Because of low resistance levels (\approx 5%), trimethoprim/sulfamethoxazole (TMP/SMX) remains the therapy of choice worldwide. A recent study encompassing data from Europe, Latin America, and North America indicates that the level of resistance to TMP/SMX is 3.8%; however, previous studies indicate that the level is higher in Latin America than North America (5,6). Although surveillance studies are few, resistance to TMP/SMX appears to be emerging, and recent in vitro modeling studies have shown that combination therapies of TMP/SMX plus ciprofloxacin and TMP/SMX plus tobramycin exhibit a greater killing capacity then TMP/SMX alone (7,8).

S. maltophilia exhibits an array of mechanisms that singularly or collectively contribute to its multidrug resistance status. Intrinsic resistance includes inducible efflux pumps (2) and multiple β -lactamase expression (1) but not mutations in the quinolone resistance–determining region (9). In addition, S. maltophilia can acquire resistance through integrons, transposons, and plasmids (10). Recently, class 1 integrons have been characterized from S. maltophilia strains isolated in Argentina and Taiwan, which indicates that they contribute to TMP/SMX resistance through the sul1 gene carried as part of the 3' end of the class 1 integron (10).

In addition to class 1 integrons, other mobile elements are associated with *sul* genes. For example, *Vibrio cholerae* serogroup 0139 is resistant to several antimicrobial agents, including SMX, and it has been recently shown that the *sul*2 gene was part of a cluster located on a novel genetic

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element of the integrative conjugative element group named SXT. The resistance genes harbored by SXT are embedded in a composite transposon-like structure and were probably acquired recently (11). Within this antimicrobial drug resistance region, an insertion element common region (ISCR) sequence, ISCR2, is adjacent to a *sul2* gene that moves by 1-ended transposition. Thus, the possibility exists that *sul2* genes can transfer intra- and intergenerically, including into *S. maltophilia*. Herein, we describe the molecular characterization of an international collection of *S. maltophilia* isolates and determine their mechanism of resistance to TMP/SMX, including the first report of *sul2* genes and the first description of insertion element common region (ISCR) elements carried in *S. maltophilia*.

Methods and Materials

Bacterial Strains

During 1998–2003, a total of 1,744 *S. maltophilia* isolates collected worldwide were forwarded to the SENTRY Program (Europe, USA, and Australia) and tested for antimicrobial drug susceptibility. A TMP/SMX resistance phenotype was demonstrated for 71. From these isolates, 25 nonclonal strains from patients in North America, Latin America, and Europe were analyzed by using molecular methods together with 30 representative isolates that were TMP/SMX-susceptible. Isolates were identified by using the Vitek System and confirmed by using API20NE (bioMérieux, Hazelwood, MO, USA).

Susceptibility Methods

Isolates were tested for susceptibility to TMP/SMX according to procedures of the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS]) (12,13) by using broth microdilution methods (TREK Diagnostics, Cleveland, OH, USA). MIC results were confirmed with TMP/SMX. Etests were performed according to the manufacturer's directions (AB Biodisk, Solna, Sweden).

Molecular Materials

PCR primers were purchased from Sigma-Genosys Ltd. (Pampisford, UK) and are listed in the Table. General reagents for DNA manipulation were obtained from Invitrogen (Groningen, the Netherlands). All other reagents were obtained from Sigma Chemical Co. or BDH (both of Poole, England, UK).

Strain Typing

Clonality among the S. maltophilia isolates was assessed by pulsed-field gel electrophoresis (PFGE) fol-

lowed by *Xba*I digestion of genomic DNA. This assessment was conducted according to the standard 1-day protocol (*16*).

Plasmid Isolation

Bacterial plasmids were isolated by the alkaline lysis method described by Grinsted and Bennett (17). Essentially, an overnight 10-mL culture was centrifuged (12,000× g) and suspended in water (250 µL) before 200 µL of lysis solution (0.2 mol/L NaOH, 1% sodium dodecyl sulfate [SDS]) was added. After lysis, 125 µL of neutralizing solution (0.3 mol/L potassium acetate, 1 mmol/L EDTA) was added. After precipitation, the suspension was centrifuged (12,000× g) and washed twice with 500 µL of a 50/50 (v/v) phenol/chloroform solution. The DNA was precipitated from the solution with the addition of 0.7 volumes of iso-amyl alcohol. The DNA/RNA pellet was washed twice in 1 mL 70% ethanol before being dried. The DNA was dissolved in 30 µL with 0.1 U RNase.

Southern Hybridization

ISCR and *sul*2 PCR amplicons generated with primers CRF/CRFF-r were labeled with P³²-CTP by random primer extension by using a commercially available kit (Stratagene, Amsterdam, the Netherlands) according to the manufacturer's instructions. Unincorporated nucleotides were removed by passing the labeled DNA through a Sephadex column (Nick column, Pharmacia Bio-tech, Uppsala, Sweden).

Agarose gels used for Southern transfer were denatured for 45 min in denaturing solution (0.5 mol/L NaOH, 1.5 mol/L NaCl) before being neutralized in 0.5 mol/L Tris-HCl, pH 7.5, 1.5 mol/L NaCl for 30 min. DNA was then transferred to Hybond (Amersham, Buckinghamshire, UK) nylon membrane by vacuum by using a custom-made Southern blotting apparatus. The nylon filter was prehybridized for at least 2 h with a blocking solution (6× SSC [1× SSC is 0.15 mol/L NaCl plus 0.014 mol/L sodium citrate], 0.1% [w/v] polyvinylpyrrolidone 400, 0.1% Ficoll [v/v], 0.1% bovine serum albumin, 0.5% SDS, 150 µg/mL denatured calf thymus DNA) at 65°C. The labeled denatured probe was then added to the solution and incubated overnight at 65°C. Finally, the filter was washed (300 mL $2 \times$ SSC, 0.1% [w/v] SDS followed by 0.1× SSC 0.1% SDS) at 65°C. Autoradiographic images were recorded on Hyperfilm-MP (Pharmacia Bio-tech), which was exposed overnight with intensifying screens.

PCR Analysis

The presence of class 1 integrons in each strain was assessed by using class 1 specific primers. Gene cassettes embedded within the class 1 integrons were determined by using primers listed in the Table. Isolates were also screened for *sul*1, *sul*2, and *sul*3 by using sull-F and -R, sul2-F and -R, and sul3-F and -R, respectively. Seven positive class 1 integron PCR products were chosen randomly, extracted from agarose gels after size separation, and sequenced with IntF, IntR, and custom-made oligonucleotide primers (Table).

The presence of ISCR elements in each strain was also determined by using primers CRF/CRFF-r designed to amplify the same 700-bp fragment internal to the open reading frames (ORFS) of ISCR1-5 (Table). Full-length ISCR2 elements were amplified with primers designed to target the ends of ISCR2. Primers used to amplify genes often associated with ISCR2 or ISCR3 are also given (Table). Because *dhfr* genes are associated with ISCR elements, we also performed molecular analysis of them.

PCRs were conducted in a final volume of 20 μ L by using 10 μ L ABgene Expand Hi-fidelity Master Mix (ABgene House, Surrey, UK). Primers were used at final concentrations of 10 μ mol/L, and 1 μ L of an overnight bacterial culture (optical density 1.0 at 600 nm) was added as source of DNA template. The cycling parameters were as follows: 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 55°C for 1 min, and 68°C for 1–4 min, depending on the sequence to be amplified, and ending with a 5-min incubation at 68°C.

DNA Sequencing and Analysis

Sequencing was conducted on both strands by the dideoxyl-chain termination method with a Perkin-Elmer Biosystems 377 DNA sequencer (Perkin-Elmer, Waltham, MA, USA). Sequence analysis was performed with the Lasergene DNASTAR software package (SelectScience Ltd., Bath, UK). Sequence alignments were conducted with the ClustalW program (www.ebi.ac.uk/clustalw) and the PAM 250 matrix.

The sequence of ISCR2, together with the adjacent *sul*2 region and the novel ISCR9 and ISCR10, has been deposited in GenBank. The genetic locus ISCR2-*glmM/sul*2 from isolates 5232, 4647, 3800, and 2107 has been attributed the accession nos. AM182031, 182030, 182029, and 181666, respectively. ISCR9 and ISCR10 have been given the numbers AM182033 and AM182032, respectively.

Table. Oligonucle	otide primers used in this study, Cardiff, 2007			
_		GenBank		
Primer	Sequence $(5' \rightarrow 3')$	accession no.	Target	Reference
Ina-F	GCCTGTTCGGTTCGTAAGCT		intl	(14)
Int-R	CGGATGTTGCGATTACTTCG		intl	(14)
sul1-F	ATGGTGACGGTGTTCGGCATTCTGA		sul1	(15)
sul1-R	CTAGGCATGATCTAACCCTCGGTCT		sul1	(15)
sul2-F	GAATAAATCGCTCATCATTTTCGG	AJ289135	sul2	(15)
sul2-R	CGAATTCTTGCGGTTTCTTTCAGC	AJ289135	sul2	(15)
aacA4-F	AACTTGCGAGCGATCCGATG		aacA4	(14)
aacA4-R	ATGTACACGGCTGGACCATC		aacA4	(14)
aacA7-F	AATGGATAGTTCGCCGCTCG		aacA7	This study
aacA7-R	TTCCGGAAGCAGCGTACTTG		aacA7	This study
CRF	CACTWCCACATGCTGTKKC	AF231986	All ISCR	This study
CRF-r	GMMACAGCATGTGGWAGTG	AF231986	All ISCR	This study
CRFF	GGRYGCAACGSCTCAAGCG	AF231986	All ISCR	This study
CRFF-r	CGCTTGAGSCGTTGCRYCC	AF231986	All ISCR	This study
LECR2	CACTGGCTGGCAATGTCTAG	AF231986	ISCR2	This study
RECR2	CTTTGGACCGCAGTTGACTC	AF231986	ISCR2	This study
FloF	TCGACATCCTGGCTTCACTG	AF231986	floR	This study
FloR	ATTACAAGCGCGACAGTGGC	AF231986	floR	This study
dfra20f	GGGAAACACCGAGAAATGGG	AJ605332	dfrA20	This study
dfrA20R	TTCTTCTTCCCATTCTCCCC	AJ605332	dfrA20	This study
dfrA9F	CAGATTCCGTGGCATGAACC	X57730	dfrA9	This study
dfrA9R	GACCTCAGATACGAGTTTCC	X57730	dfrA9	This study
dfrA10F	TGTAGCGCGTGGTGTAAACG	AY055428	dhfr10	This study
dfrA10R	ACGTCTACGTGAGTATCCGC	AY055428	dhfr10	This study
strAF	TCTGTCGCACCTGCTTGATC	AY055428	strA	This study
strAR	CATTGCTGATGAACTGCGCG	AY055428	strA	This study
tetAF	CGCTGTTTGTGATTACACCC	AJ250203	tetA	This study
tetAR	CAGCGAGATGCGATATATCC	AJ250203	tetA	This study
glmmR	GAGTCAACTGCGGTCCAAAC	AJ289135	gImM	This study
glmmF	ACGGTATTCGTGGCAAAGCC	AJ289135	glmM	This study

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Results

TMP/SMX MICs

TMP/SMX MICs separated the isolates into an obvious bimodal distribution. The TMP/SMX-resistant isolates possessed MICs >32 mg/L, whereas the sensitive controls used as molecular comparators possessed TMP/SMX MICs ranging from 0.5 to 2 mg/L (Online Appendix Table, available from http://www.cdc.gov/EID/content/13/4/ 559-appT.htm).

Detection and Determination of Class 1 Integrons

Of the 25 TMP/SMX-resistant S. maltophilia isolates that we analyzed, 17 possessed the sul1 gene as part of the 3' end of a class 1 integron. None of the TMP/SMX-susceptible S. maltophilia isolates yielded positive sull PCR products. PFGE analysis (data not shown) showed that only 2 isolates (9189 and 12221 from Chile) are clonally related (online Appendix Table). To our knowledge, this is the first report of sul1-positive S. maltophilia isolates from North America and Europe. The sul1-positive isolates are widespread, being from Europe, North America, and South America. Most (5) were isolated from Brazil. The integrons associated with the sul1 gene vary in size; however, when 2 strains were isolated from the same country (e.g., 3438 and 3444, 9189 and 12221, and 98 and 14469), they possessed integrons of the same size, despite not being clonally related (Online Appendix Table). Seven of these integrons were randomly selected to examine their gene cassettes. The genetic context of the class 1 integrons and procured gene cassettes are shown in Figure 1. Strains 1893 (Germany) and 9431 (Brazil) possessed only the int and sul/qac genes. The class 1 integrons from strains 4891 (USA), 9189 (Chile), and 12221 (Chile) contained an embedded aacA4 gene cassette. The 2 Mexican strains (3438 and 3444) contained 2 aminoglycoside-modifying genes (*aacA7* and *aadA5*) and an unknown ORF (Figure 1) vet were clonally unrelated, as judged by PFGE profiling. None of the integrons were the same as those characterized from strains isolated from Argentina (10).

Detection and Location of sul2 Genes

All 55 isolates (both TMP/SMX resistant and sensitive) were screened for *sul2* genes with the primers listed in the Online Appendix Table. Nine of the isolates gave PCR products for *sul2*. None of the TMP/SMX-susceptible *S. maltophilia* isolates displayed positive *sul2* PCR products. Sequence analysis showed 100% identity with previous *sul2* sequences.

Given that *sul*² is normally located on medium-tolarge sized plasmids, plasmids were isolated and characterized for *sul*² carriage. Plasmid DNA was prepared from each isolate and used as a template for PCRs by using the sul2 primer detection set. In every case, a product of the size expected of sul2 sequence amplification was obtained. The purity of each plasmid preparation was evaluated by attempted PCR amplification of the host cell chromosomal gyrA gene. In no case was an amplification product obtained when plasmid DNA was used as template; in contrast, a gyrA amplification product of the correct size was obtained from genomic DNA. These data were later confirmed by Southern hybridization that used the labeled sul2 gene as a probe (data not shown). Unsurprisingly, in most cases sul2 was found on a large plasmid of ≈ 120 kb; however, in 2 of 9 sul2-positive isolates, sul2 gene was chromosomally encoded.

Detection of ISCR Elements in TMP/SMX-sensitive and -resistant Strains

The sul2 gene and *dhfr* genes are often found on plasmids and in close association with class 1 integrons or ISCR mobile genetic elements (10,15,18,19). Accordingly, we investigated the 55 S. maltophilia isolates for ISCR elements. Seven of the 25 TMP/SMX-resistant isolates yielded PCR products of the expected size (\approx 700 bp) when the ISCR specific primers CRF/CRFF-r were used, and 6 of 23 TMP/SMX-sensitive S. maltophilia isolates also yielded the correct-sized amplification products.

To determine whether the locations of the ISCR sequences in the *S. maltophilia* isolates are chromosomal or plasmid mediated, plasmid DNA was prepared from each isolate and used as a template for ISCR-PCR and Southern hybridization analysis in a similar manner as described for *sul*2. In every case, a product of the size expected of ISCR sequence amplification was obtained. Hence, in those isolates that possess an ISCR element, the element is located on a plasmid (data not shown). The PCR



Figure 1. Schematic diagram of class 1 integrons from *Stenotrophomonas maltophilia* isolates. A) isolates 1893 and 9431; B) isolates 489, 9189, and 12221; C) isolates 3438 and 3444. Arrows depict direction of transcription, and shaded boxes represent gene cassettes found within the integron. The dark circles represent the 59-bp region immediately 5' to the incorporated gene cassette.

ISCR amplification products were recovered, purified, and ligated into the cloning vector, PCR-Topo-2.1 (Invitrogen) and recombinant plasmids were recovered by transformation of *Escherichia coli* DH5α. One clone from each transformation was chosen for further study.

Sequence analysis showed that 5/7 amplicons obtained from TMP/SMX-resistant *S. maltophilia* isolates were identical to the equivalent sequence of IS*CR2*; the other 2 amplicons were identical to that of IS*CR3* (Online Appendix Table). IS*CR2* sequences were identified in isolates originating from North and South America, as well as from Europe. In contrast, the IS*CR3* sequence was identified only in isolates that originated from Spain.

The ISCR-like elements carried by the sensitive isolates, while clearly related to ISCR1–5, differed markedly from known ISCR sequences (15). Two variants were found, which we have designated ISCR9 and ISCR10. The putative amino acid sequences of ISCR9 and ISCR10 are \approx 95% identical to each other and display 30%, 48%, and 74% identity to ISCR2, ISCR,3 and ISCR5, respectively (Online Appendix Figure, available from http://www.cdc. gov/EID/13/4/content/559-appG.htm). These novel ISCRs are harbored in isolates from several different regions, including South American countries, the United States, and Turkey (Table).

Identification of Resistance Genes and Sequences Adjacent to ISCR Elements

ISCR2 is often associated with various antimicrobial resistance genes, not least, genes mediating TMP/SMX resistance (Figure 2) (15). These and other genes normally associated with ISCR2 were therefore analyzed; these included *dhf*rA10, *dhf*rA9, *dhf*rA20, *flo*R, *tet*R, *str*A, *sul*2, and *glmM* encoding a truncated phosphoglucosamine mutase. Pairs of oligonucleotides were used (Table) to genetically characterize all those *S. maltophilia* isolates that possessed an ISCR element.

The *flo*R gene was detected in isolates 2139 and 2170 (which also contains ISCR2) from Turkey and the United States, respectively, and in isolates 12044 and 12049 (which also contains ISCR3) from Spain. A truncated *glmM* allele ($\Delta glmM$) was detected in all ISCR2-containing isolates, and *sul*2 was found in all ISCR2- and ISCR3- containing isolates. The *dhfr, tetR*, and *strA* genes were not detected.

Linkage of the ISCR element to $\Delta glmM$, sul2, or floR was then investigated by PCR analysis, i.e., the oligonucleotide pair CRFF/sul2F is expected to generate a product if the ISCR sequence is close to sul2 and downstream of it (Figure 2). Using this strategy, we found that ISCR2 was linked to $\Delta glmM$ and sul2 in all isolates that possess ISCR2. The floR gene was also found to be linked to ISCR2, on the opposite side from $\Delta glmM$ and sul2, in



Figure 2. Genetic context of various insertion element common region (ISCR2) elements. Schematic of various GenBank sequences that harbor the ISCR2 element. Open reading frames (ORFs) are depicted as open boxes with the direction of transcription of the various ORF indicated with arrows. The ORF of the ISCR2 transposase is highlighted, and the limits of the ISCR2 element are shown with vertical lines. The ISCR2 element consists of an ORF of 1491 bp together with ≈120 bp of upstream sequence and ≈240 bp of downstream sequence. The ISCR2 element identified in this study associated with the floR resistance gene is amplified to show primer sites and positions of the putative orilS and terIS of the ISCR2 element. The bold arrow indicates the direction of rolling circle replication of the ISCR2 element. GenBank accession numbers of the various sequences are indicated. S. enterica, Salmonella enterica; S. maltophilia, Stenotrophomonas maltophila. *This study.

isolates 2139 and 2170 (Figure 2). Linkage of ISCR3 to either *sul2* or *floR* was not demonstrated.

Discussion

We report *sul*2 genes being present in *S. maltophilia* and contributing to TMP/SMX resistance. In most cases, *sul*2 was carried on large plasmids (\approx 120 kb), but as judged by Southern hybridization data, a few appear to be chromosomally encoded. This study also supports the findings of Barbolla et al. that *sul*1 present in *S. maltophilia* is associated with class 1 integrons (*10*). Herein, we have characterized *S. maltophilia sul*1 genes from North America, South America, Spain, Turkey, Italy, and Germany, and observed that all of them were associated with class 1 integrons.

Most studies of the location and dissemination of *sul*2 genes have concentrated on *Enterobacteriaceae*, such as *E*.

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coli and Salmonella enterica. A recent study by Antunes et al. found sul1, sul2, or sul3 genes in most Portuguese isolates (18); 24 of 200 isolates contained both sul1 and sul2. sul2 has also recently been identified in S. enterica from Brazil (20). Similar results have been reported from E. coli urinary tract isolates in which $\approx 26\%$ of strains possessed both sul1 and sul2 genes (21). A biased study examining TMP/SMX-resistant E. coli recently reported that 15 of 20 isolates possessed sul2 and that 6 of those also carried sul1 on a class 1 integron (14). Additional studies of E. coli have shown the intercontinental predominance of sull through class 1 integrons (22). A study by Pei et al. demonstrated the correlation of anthropogenic activity with the presence of sul genes in environmental samples (23). However, none of the studies demonstrated the genetic origin of the sul2.

In addition to sul genes associated with plasmids and class 1 integrons, we investigated whether the S. maltophilia isolates possessed ISCR elements and whether these could be linked to *dhfr* or *sul* genes, as has been shown (18). Of the 25 TMP/SMX-resistant isolates, 6 harbored sul2 linked to ISCR2. However, we could not detect any sul3 genes. In the isolates with ISCR2, the element was directly linked to a deleted version of a phosphoglucosamine mutase gene, Δglm M, as has been reported on other occasions (Figure 2). This arrangement is identical to those of 5 other sequences in the EMBL database, in E. coli isolated from cattle in France and Germany (24), in the plasmid pRVS1 isolated from a strain of Vibrio salmonicida from Norway, in a plasmid from a strain of S. enterica isolated in Japan, and on the chromosome of Shigella flexneri isolated in the United States (18,24). In all cases, $\Delta glmM$ and sul2 are linked to the end of ISCR2 that accommodates the IS91 oriIS equivalent (Figure 2). The dual arrangement of Δglm M and sul2 is also found in plasmids of marine psychotrophic bacteria isolated in Norway (GenBank accession no. AJ306553/4), but in these cases the ISCR2 element appears not to be present.

Two of the isolates harbored a copy of the *flo*R gene immediately upstream of a copy of IS*CR2* (Figure 2), an arrangement identical to that reported on plasmids found in isolates of *E. coli* from cattle in France and Germany (24). The *S. maltophilia* isolates investigated in this study came from Turkey and the United States. Two isolates from Spain also carry the *flo*R gene but not IS*CR2*. Instead, the isolates possess copies of IS*CR3*, which do not appear to be linked to *flo*R. The finding of florfenicol-resistant traits on plasmids in different bacterial species from different countries highlights the wide geographic spread of this resistance mechanism. The location of *flo*R next to IS*CR2* is such that it is possible, if not probable, that the resistance gene can be cotransposed with the IS*CR* element.

The findings within this study are important for several reasons. First, this is, to our knowledge, the first report of ISCR elements being found in S. maltophilia isolates. In 6 cases, these were linked to sul2 genes responsible for the TMP/SMX-resistant phenotype. Moreover, these isolates were unrelated strains found in different countries. Second, since TMP/SMX is the mainstay therapy for S. maltophilia infections, the mobilization of sul genes by means of class 1 integrons and ISCR elements is likely to increase with TMP/SMX consumption. Third, most sul2 genes in this study have been found on plasmids, and sul2-containing plasmids can potentially confer an increase in bacterial "fitness" (25). As yet, such phenomena have only been explored in Enterobacteriaceae, and it has yet to be established whether sul2-carrying plasmids have such an additive effect in S. maltophilia or for that matter, other nonfermenting gram-negative bacilli.

These data suggest that microbiology laboratories need to carefully monitor *S. maltophilia* TMP/SMX resistance, which has the potential to increase by means of mobile elements. We also advocate the continued international surveillance of antimicrobial drug resistance that may act as early warning systems for this kind of resistance. Furthermore, yearly monitoring with molecular probes is advisable.

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Dr Toleman is currently working as a research fellow at the Medical School, Cardiff University, Wales. His interest is the dissemination of antimicrobial-drug–resistant genes among clinical and environmental bacteria.

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SENTRY		TMP/SMX MIC	Integron size		Plasmid-mediated				
isolate	Country	Etest	(kb)	<i>sul</i> 1	sul2	Plasmid	sul2	ISCR	
7666	Argentina	>32	4.5	+	_	_	_	_	
40-9046	Argentina	<0.5	_	-	-	-	-	-	
40–531	Argentina	<0.5	_	-	-	-	-	-	
6147	Brazil	2	_	-	-	-	-	-	
98	Brazil	>32	1.0	+	-	-	_	-	
3932	Brazil	>32	4.5	+	+	+	+	-	
4647	Brazil	>32	_	-	+	+	+	-	
9431	Brazil	>32	0.4	+	-	-	_	-	
12357	Brazil	>32	1.2, 1.5	+	-	-	-	-	
14469	Brazil	>32	1.0	+	+	+	+	-	
48–1528	Brazil	<0.5	-	-	-	-	-	-	
48–115	Brazil	<0.5	-	-	-	-	-	ISCR9	
48–1528	Brazil	<0.5	-	-	-	-	-	-	
57–1526	Brazil	<0.5	-	-	-	-	-	-	
57–303	Brazil	<0.5	-	-	-	-	-	_	
12221	Chile	>32	1.0	+	-	-	-	-	
9189	Chile	>32	1.0	+	-	+	-	_	
43–2380	Chile	<0.5	-	-	-	-	-	ISCR9	
43–2092	Chile	<0.5	-	-	-	-	-	-	
43–1971	Chile	<0.5	-	-	-	-	-	-	
3438	Mexico	>32	3.0	+	-	-	-	-	
3444	Mexico	>32	3.0	+	-	-	_	-	
45–11382	Mexico	<0.5	-	-	-	-	-	-	
45–6651	Mexico	<0.5	_	-	-	-	_	-	
7618	Venezuela	>32	4.0	+	-	-	-	-	
49–6147	Venezuela	2	_	-	-	-	_	-	
49–1036	Venezuela	<0.5	_	-	-	-	_	ISCR10	
49–3886	Venezuela	<0.5	-	-	-	-	-	-	
345	Canada	>32	1.4	+	-	-	_	-	
38–1086	Canada	<0.5	-	-	-	-	-	-	
38–3673	Canada	<0.5	-	-	-	-	-	-	
867	USA	>32	_	-	-	+	_	-	
4891	USA	>32	1.0	+	-	+	-	_	
2170	USA	>32	-	-	+	+	+	ISCR2	
4568	USA	2	_	-	-	-	_	-	
1246	USA	1	-	-	-	-	-	_	
1113	USA	1	_	-	-	-	_	-	
1696	USA	1	-	-	-	-	-	_	
15–6279	USA	<0.5	-	-	-	-	-	-	
15–6290	USA	<0.5	_	-	-	-	_	IS <i>CR9</i>	
4225	Belgium	>32	-	-	-	-	-	_	
11131	France	1	_	-	-	-	_	-	
12876	France	>32	_	-	+	+	+	-	
1893	Germany	>32	0.4, 0.9	+	-	-	-	-	
5232	Germany	>32			+	+	+	ISCR2	
2597	Italy	>32	1.3, 1,5	+	-	-	-	-	
75–12113	Italy	<0.5	-	-	-	-	-	-	
75–16066	Italy	<0.5	-	-	-	-	-	-	
12044	Spain	>32	-	-	+	+	-	IS <i>CR3</i>	
12049	Spain	>32	0.4, 3.0	+	-	-	-	IS <i>CR3</i>	
66–12258	Spain	<0.5	-	_	_	-	-	-	
2139	Turkey	>32	_	_	+	+	-	ISCR2	
3800	Turkey	>32	-	_	+	+	+	ISCR2	
14263	Turkey	>32	1.0	+	_	_	_	ISCR2	
69–2333	Turkey	<0.5	_	_	_	_	_	ISCR10	
69–4959	Turkey	<0.5	_	-	_	_	-	ISCR10	

Appendix Table. Origin, TMP/SMX susceptibility profiles, and genetic characterization of *sul* and IS*CR* elements in *Stenotrophomonas maltophilia* isolates*