ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2006, p. 799–802 Vol. 50, No. 2
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.2.799–802.2006

In117, an Unusual In0-Like Class 1 Integron Containing CR1 and *bla*$_{\text{CTX-M-2}}$ and Associated with a Tn*21*-Like Element

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Received 15 June 2005/Returned for modification 31 July 2005/Accepted 7 November 2005

An unusual In0-like class 1 integron containing a common region that includes the putative recombinase gene named orf513 (CR1) and *bla*$_{\text{CTX-M-2}}$ was characterized from *Escherichia coli*. The integron contained an unusual gene cassette array, *estX-aadA1*, embedded between the 5′-conserved segment (5′-CS) and 3′-CS1 regions and was flanked by *mer*-Tn*21* sequences downstream of the *tni* truncated module. This element constitutes one of the few examples of CR1-bearing class 1 integrons that has been fully characterized.

The CTX-M enzymes are among the most widespread extended-spectrum β-lactamases of Ambler class A (5). Five clusters of CTX-M β-lactamases (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25) have been described on the basis of their amino acid sequences (http://www.lahey.org/studies/webt.htm). Chromosomal genes from *Kluyvera* species have been identified as progenitors of each CTX-M group, and mobilization seems to have occurred by the association of these genes to CR1, IS*ECp1*, or phage-related elements (3, 5, 16, 25). The *bla*$_{\text{CTX-M-2}}$ and *bla*$_{\text{CTX-M-9}}$ genes have been mainly associated with class 1 integrons containing CR1. Their backbone structure consists on the 5′-conserved segments (5′-CS) and 3′-CS flanking variable gene cassette arrays, CR1, several antibiotic resistance genes that do not resemble gene cassettes, and a second partial copy of the 3′-CS designated 3′-CS2 (2, 5, 24). The sequences upstream of the 5′-CS and beyond the second copy of *qacEΔ1* at the 3′-CS2 have been described only for In6 and In34 (19). The aim of this work was to characterize the genetic environment of *bla*$_{\text{CTX-M-2}}$ in *Escherichia coli* strain VS27, one of the few CTX-M-2-producing isolates described in Spain.

E. coli VS27 (resistant to β-lactams and streptomycin, sulfonamide, tetracycline, nalidixic acid, and ciprofloxacin) was recovered from the feces of a healthy volunteer without recent hospitalization or antibiotic exposure in 2003 (29). Transfer of *bla*$_{\text{CTX-M-2}}$ by broth and filter-mating methods using *E. coli* BM21R (nalidixic acid and rifampin resistant, lactose fermentation positive, and plasmid free) or *E. coli* HB101 (kanamycin and azide resistant, lactose fermentation negative, and plasmid free) as the recipient strain was unsuccessful. The whole-plasmid profile determined by the Kado and Liu method using *E. coli* V517 and *E. coli* NCTC 50192 as control strains for the estimation of plasmid sizes consisted of three plasmids of 70, 40, and 5 kb and three plasmids of less than 3 kb (27). Hybridization of plasmid DNA (26) with an intragenic *bla*$_{\text{CTX-M-2}}$ probe labeled and detected by ECL kits according to the manufacturer's instructions (Amersham Life Sciences, Uppsala, Sweden) was negative.

Although *bla*$_{\text{CTX-M-2}}$ has been previously associated with class 1 integrons bearing CR1, these elements have been only partially characterized (2, 3, 23). An overlapping PCR assay based on the sequence of In35 containing *bla*$_{\text{CTX-M-2}}$ and Tn*21*, often associated with class 1 integrons, was designed (GenBank accession numbers AY079169 and AF071413) (3, 12, 13) (Fig. 1). PCR assays were performed in volumes of 50 μl with a mixture containing 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate, 0.1 μM of each primer, and 1.5 units of *Taq* DNA polymerase (AmpliTaq Gold; PE Applied Biosystems, Norwalk, Conn.) for 12 min at 94°C and for 35 cycles at 94°C (1 min), 56 to 65°C (1 to 2 min), and 72°C (1 to 3 min) followed by a final step for 10 min at 72°C for standard PCR assays and with a mixture containing 2.5 mM $MgCl_2$, 0.1 μM of each primer, and 2.5 units of Takara LA *Taq* polymerase (Takara Bio Inc, Shiga, Japan) for 1 min at 94°C and for 35 cycles of 96°C (20 s), 60°C (1 min), and 72°C (3 to 5 min) followed a final step for 10 min at 72°C for long PCRs (>3 kb). Amplified products were purified using the QIAquick PCR purification kit (QIAGEN) and sequenced on an ABI Prism 377 automated sequencer (PE Applied Biosystems). The oligonucleotide sequences used in PCR assays are listed in Table 1.

Analysis of the 15,882-bp sequence between inverted repeats of Tn*402* located upstream of *intI1* and downstream of *tniA* revealed the presence of an integron belonging to the In0 group that we called In117. The 5′-CS region includes a copy of the integrase *intI1* with a P_c promoter identical to that of In1 in R46 (GenBank accession number AY046276), consisting of TGGACA(−35) and TAAACT(−10) hexamers separated by 17 bp (22). This P_c promoter is of intermediate strength in In1, and although it has been described less frequently than P_c promoters of weak or strong strength, it is increasingly being found in specific class 1 integrons carrying *bla*$_{\text{IMP}}$, *bla*$_{\text{VIM}}$, or *bla*$_{\text{GES}}$ (10, 28, 30). A gene cassette array, *estX-aadA1*, within the 5′-CS–3′-CS1 region was identified. The deduced amino acid sequence of the *estX* gene displayed 90% amino acid

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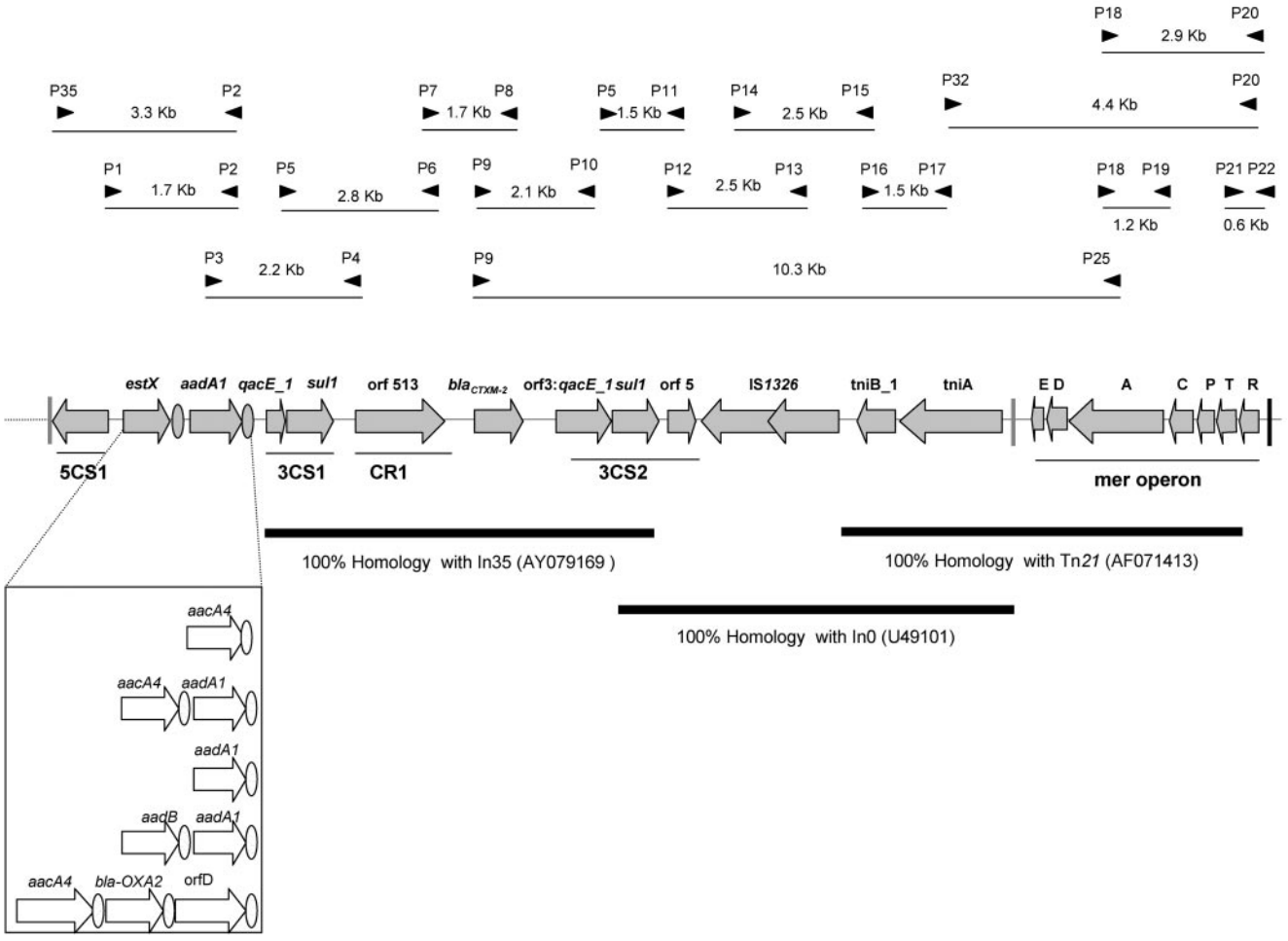

FIG. 1. Schematic representation of the $bla_{CTX\text{-}M\text{-}2}$ genetic loci. A comparison with other gene array cassettes located within the 5′-CS–3′-CS1 region described to date is represented at the bottom of the left side (2). The location of the primers used for the identification of the element by PCR-overlapping assay are represented with black arrows. Vertical bars symbolize inverted repeats of the integron (gray) or Tn*21* (black). Gray shaded open reading frames represent the 20,032-bp region fully sequenced, with 15,882 bp corresponding to In117. Circles represent 59-bp elements of the corresponding gene cassettes. GenBank accession numbers are in parentheses.

identity with *sat-1* of Tn*1825* conferring resistance to streptothricin, 40% amino acid identity with proteins annotated as putative esterases or hydrolases of the α/β fold superfamily, and 42% amino acid identity with a protein encoded in *E. coli* multiresistance plasmids. The *estX* gene was initially considered to be a *sat* cassette because of its similarity with *sat-1*; however, Partridge and Hall have demonstrated that *sat-1* resulted from the fusion of *estX* and *sat-2* genes, suggesting a change in the nomenclature of these genes (18). The *estX-aadA1* gene cassette combination has not been previously linked to CTX-M-2-producing isolates, although it has been associated with class 1 integrons from *Shigella sonnei* clinical strains, and *estX* has been found in different class 1 and class 2 integrons from community isolates at different locations (1, 4, 8).

The 5,585-bp region from 3′-CS1 to the second copy of *qacEΔ1* in 3′-CS2 showed 100% homology with that of In35 (GenBank accession number AY079169). The extent of 3′-CS2 was identified as a 6,900-bp sequence with 100% homology with class 1 integron In0 (GenBank accession number U49101). This sequence includes the typical 3′-CS (*qacEΔ1*, *sul1*, and orf5) followed by the insertion sequence IS*1326*, a member of the IS*21* family, and a truncated *tni* module of Tn*402* (7). Although several members of the IS*21* family are widely distributed, IS*1326* remains associated with the class 1 integron lineage In0-In2-In5 (7, 19, 22), which differ one from another in the promoter of *intI1* and in the truncated *tni* module sequences originating from the insertion of IS*1326* and further deletion events. Tn*21* sequences (left inverted repeats and *tnpR*) upstream of *intI1* were not detected. Interestingly, amplification with primers specific for the *mer* locus and inverted repeats of Tn*21* and the integron showed the presence of *mer*-Tn*21* sequences downstream of *tniA* (Fig. 1) (12, 32).

Our results revealed the presence of $bla_{CTX\text{-}M\text{-}2}$ in a defective transposon derivative of the Tn*402* family and constitute, besides In34 and In6, one of the few examples of class 1 integrons containing CR1 in which the structure beyond the 3′-CS2 has been established (19). In0, In2, and In5 are Tn*402* derivatives located in plasmids and/or transposons, often in mercury resistance transposons such as Tn*21*. These trans-

TABLE 1. Oligonucleotides used in this study

Primer no.	Primer	Sequence	Positions	GenBank accession no.	Reference or source
1	5′-CS	5′-GGCATCCAAGCAGCAAG-3′	5298–5314	AF071413	11
2	3′-CS	5′-AAGCAGACTTGACCTGAT-3′	6306–6289	AF071413	11
3	aadA1F	5′-GCTGGCCGTGCATTTGTACG-3′	5487–5506	AF071413	This study
4	ORF513rF1R	5′-GAGCTCTGCACCATCCCAC-3′	527–507	AY079169.1	This study
5	qacEΔ2	5′-ATCGCAATAGTTGGCGAAGT-3′	6383–6392	AF071413	This study
6	ORF513-4F	5′-CTCGCTTGAGGCGTTGCAT-3′	2106–2088	AY079169.1	This study
7	ORF513-4R	5′-ATGCAACGCCTCAAGCGAG-3′	2088–2106	AY079169.1	This study
8	CTX-M-2R/P2b	5′-TCCCGACGGCTTTCCGCCTT-3′	3655–3637	AY079169.1	31
9	CTX-M-2F/P3	5′-ATGATGACTCAGAGCATTCG-3′	2823–2842	AY079169.1	31
10	qacEΔ1B	5′-CAAGCTTTTGCCCATGAAGC-3′	5050–5031	AY079169.1	This study
11	orf5-R	5′-AGTTCTAGGCGTTCTGCG-3′	8157–8140	AF071413	This study
12	orf5-F	5′-CGATATCGACGAGGTTGTGC-3′	7712–7730	AF071413	This study
13	IS1326-F	5′-TACCGGGTCTTATGACCGAGT-3′	10357–10337	AF071413	This study
14	IS1326-R	5′-ACTGTCATAGCGGTTCACGTT-3′	9141–9161	AF071413	This study
15	tniBΔ1F	5′-ATCATCGACCTGTCCCACCT-3′	13201–13182	AF071413	This study
16	tniBΔ1R	5′-AGGTGGGACAGGTCGATGAT-3′	13182–13201	AF071413	This study
17	tniAF	5′-TCGTGCGGAGATCATCAGTCC-3′	14821–14801	AF071413	This study
18	merA1	5′-ACCATCGGCGGCACCTGCGT-3′	17597–17578	AF071413	13
19	merA5	5′-ACCATCGTCAGGTAGGGGAACAA-3′	16360–16382	AF071413	13
20	merR1	5′-GCGGATTTGCCTCCACGTTGA-3′	19278–19260	AF071413	13
21	merT1	5′-CCAGGCAGCAGGTCGATGCAAG-3′	19055–19076	AF071413	13
22	Tn21IR/38	5′-GGGCACCTCAGAAAACGGAAA-3′	19669–19649	AF071413	14
23	TnpR-F[a]	5′-ATGCTATGCACCACCACGG-3′	3376–3394	AF071413	14
24	intF1	5′-GGGTCAAGGATCTGGATTTCG-3′	4774–4754	AF071413	This study
25	merA6	5′-GCCGACCAGTTGTTCCCCTACCTGACG-3′	16391–16365	AF071413	13
26	merD1	5′-CGCACGATATGCACGCTCACCC-3′	16211–16233	AF071413	13
27	merA0	5′-GTCGCAGGTCATGCCGGTGATTTT-3′	178950–17974	AF071413	13
28	merP1	5′-GGCTATCCGTCCAGCGTCAA-3′	18520–18501	AF071413	13
29	merC1	5′-CATCGGGCTGGGCTTCTTGAG-3′	18361–18351	AF071413	13
30	merC2	5′-CATCGTTCCTTATTCGTGTGG-3′	17987–18007	AF071413	13
31	IRIn2R	5′-TGGTGCAGTCGTCTTCTGAAAA-3′	15012–15033	AF071413	14
32	tniAR	5′-GGACTGATGATCTCCGCACGA-3′	14801–14821	AF071413	This study
33	IRTn21F[a]	5′-GGGTCGTCTCAGAAAACGG-3′	1–38	AF071413	This study
34	TnpR-R[a]	5′-CCGTGGTGGTGCATAGCAT-3′	3394–3376	AF071413	This study
35	IRIn2F	5′-TTTCAGAAGACGGCTGCACTG-3′	4046–4066	AF071413	14

[a] Primers 23, 33, and 34 were used in combination with primers 24 and 35 in order to characterize the 5′ end of In117 using appropriate controls.

posons are considered to be a worldwide disseminated population composed of a few variants shared by gram-negative environmental and clinical bacteria (32). The absence of a Tn*21*-like transposition module upstream of *intI1* was not surprising, since Tn*21* subgroup transposons are frequently inactivated or yield mosaic structures by exchanging transposition modules by recombination at the *res* site (15, 20, 32). The great polymorphism within the 5′-CS–3′-CS1 region of integrons carrying $bla_{\text{CTX-M-2}}$ (2) suggests recombinatorial exchange either among cassettes of different class 1 integrons or among CR1 and class 1 integrons containing the 3′-CS1 (6, 17, 19, 21).

The presence of $bla_{\text{CTX-M-2}}$ in Spain increases the diversity of $bla_{\text{CTX-M}}$ genes described in our area, already epidemic for those of CTX-M-9 ($bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-14}}$) and CTX-M-1 ($bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-10}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{CTX-M-32}}$) clusters (9; unpublished results). The genetic elements containing $bla_{\text{CTX-M-2}}$ may fuel the dissemination of this gene, as has recently occurred for carbapenemase genes in Europe (28) or $bla_{\text{CTX-M-9}}$ in Spain (unpublished results), both of which are associated with composite transposon platforms.

Nucleotide sequence accession number. The sequence for In117 was deposited in the GenBank database under accession number DQ125241.

A.V. was funded by a fellowship from the Fondo de Investigaciones Sanitarias of Spain (PI020943-2002). This work was partially supported by research grants from Ministerio de Ciencia y Tecnología of Spain (SAF 2003-09285), the European Commission (LSHM-CT-2003-503335), and the Red Española de Investigación en Patología Infecciosa (REIPI-ISCIII-C03/14).

We thank Hatch Stokes (Macquarie University, Sydney, Australia) for kindly providing us control strains for different class 1 integrons.

REFERENCES

1. **Ahmed, A. H., H. Nakano, and T. Shimamoto.** 2005. Molecular characterization of integrons in non-typhoid Salmonella serovars isolated in Japan: description of an unusual class 2 integron. J. Antimicrob. Chemother. **55:** 371–374.
2. **Arduino, S. M., M. Catalano, B. E. Orman, P. H. Roy, and D. Centrón.** 2003. Molecular epidemiology of orf513-bearing class 1 integrons in multiresistant clinical isolates from Argentinean hospitals. Antimicrob. Agents Chemother. **47:**3945–3949.
3. **Arduino, S. M., P. H. Roy, G. A. Jacoby, B. E. Orman, S. A. Pineiro, and D. Centron.** 2002. $bla_{\text{CTX-M-2}}$ is located in an unusual class 1 integron (In35) which includes Orf513. Antimicrob. Agents Chemother. **46:**2303–2306.
4. **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.
5. **Bonnet, R.** 2004. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother. **48:**1–14.
6. **Boyd, D., A. Cloeckaert, E. Chaslus-Dancla, and M. R. Mulvey.** 2002. Characterization of variant *Salmonella* genomic island 1 multidrug resistance

regions from serovars Typhimurium DT104 and Agona. Antimicrob. Agents Chemother. **46:**1714–1722.
7. **Brown, H., H. Stokes, and R. Hall.** 1996. The integrons In0, In2, and In5 are defective transposon derivatives. J. Bacteriol. **178:**4429–4437.
8. **DeLappe, N., F. O'Halloran, S. Fanning, G. Corbett-Feeney, T. Cheasty, and M. Cormican.** 2003. Antimicrobial resistance and genetic diversity of *Shigella sonnei* isolates from Western Ireland, an area of low incidence of infection. J. Clin. Microbiol. **41:**1919–1924.
9. **Hernández, J. R., L. Martínez-Martínez, R. Cantón, T. M. Coque, A. Pascual, and the Spanish Group for Nosocomial Infections (GEIH).** 2005. *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases in Spain: a nationwide study. J. Clin. Microbiol. **49:**2122–2125.
10. **Laraki, N., M. Galleni, I. Thamm, M. L. Riccio, G. Amicosante, J. M. Frère, and G. M. Rossolini.** 1999. Structure of In31, a bla_{IMP}-containing *Pseudomonas aeruginosa* integron phyletically related to In5, which carries an unusual array of gene cassettes. Antimicrob. Agents Chemother. **43:**890–901.
11. **Levesque, C., L. Piche, C. Larose, and P. H. Roy.** 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. **39:**185–195.
12. **Liebert, C., R. M. Hall, and A. O. Summers.** 1999. Transposon Tn*21*, flagship of the floating genome. Microbiol. Mol. Biol. Rev. **63:**507–522.
13. **Liebert, C. A., J. Wireman, T. Smith, and A. O. Summers.** 1997. Phylogeny of mercury resistance (*mer*) operons of gram-negative bacteria isolated from the fecal flora of primates. Appl. Environ. Microbiol. **63:**1066–1076.
14. **Mazel, D., B. Dychinco, V. A. Webb, and J. Davies.** 2000. Antibiotic resistance in the ECOR collection: integrons and identification of a novel *aad* gene. Antimicrob. Agents Chemother. **44:**1568–1574.
15. **Noguchi, N., J. Katayama, and M. Sasatsu.** 2000. A transposon carrying the gene *mphB* for macrolides 2′-phosphotransferase II. FEMS Microbiol. Lett. **192:**175–178.
16. **Oliver, A., T. M. Coque, D. Alonso, A. Valverde, F. Baquero, and R. Cantón.** 2005. CTX-M-10 linked to a phage-related element is widely disseminated among *Enterobacteriaceae* in a Spanish hospital. Antimicrob. Agents Chemother. **49:**1567–1571.
17. **Partridge, S., C. Collis, and R. Hall.** 2002. Class 1 integron containing a new cassette, *aadA10*, associated to Tn*1404* from R151. Antimicrob. Agents Chemother. **46:**2400–2408.
18. **Partridge, S., and R. Hall.** 2005. Correctly identifying the streptothricin resistance gene cassette. J. Clin. Microbiol. **43:**4298–4300.
19. **Partridge, S., and R. Hall.** 2003. In34, a complex In5 family class 1 integron containing orf513 and *dfrA10*. Antimicrob. Agents Chemother. **47:**342–349.
20. **Partridge, S., G. Recchia, H. W. Stokes, and R. Hall.** 2001. Family of class 1 integrons related to In4 from Tn*1696*. Antimicrob. Agents Chemother. **45:** 3014–3020.
21. **Partridge, S., H. Brown, and R. Hall.** 2002. Characterization and movement of the class 1 integron known as Tn*2521* and Tn*1405*. Antimicrob. Agents Chemother. **46:**1288–1294.
22. **Partridge, S., H. Brown, H. Stokes, and R. Hall.** 2001. Transposons Tn*1696* and Tn*21* and their integrons In4 and In2 have independent origins. Antimicrob. Agents Chemother. **45:**1263–1270.
23. **Power, P., M. Galleni, J. Di Conza, J. A. Ayala, and G. Gutkind.** 2005. Description of In116, the first blaCTX-M-2-containing complex class 1 integron found in *Morganella morganii* isolates from Buenos Aires, Argentina. J. Antimicrob. Chemother. **55:**461–465.
24. **Sabaté, M., F. Navarro, E. Miró, S. Campoy, B. Mirelis, J. Barbé, and G. Prats.** 2002. Novel complex *sul1*-type integron in *Escherichia coli* carrying $bla_{CTX\text{-}M\text{-}9}$. Antimicrob. Agents Chemother. **46:**2656–2661.
25. **Saladin, M., V. T. Cao, T. Lambert, J. L. Donay, J. L. Herrmann, Z. Ould-Hocine, C. Verdet, F. Delisle, A. Philippon, and G. Arlet.** 2002. Diversity of CTX-M beta-lactamases and their promoter regions from *Enterobacteriaceae* isolated in three Parisian hospitals. FEMS Microbiol. Lett. **209:**161–168.
26. **Sambrook, J, E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
27. **Threlfall, E. J., and N. Woodford.** 1995. Plasmid profile typing and plasmid fingerprinting. Methods Mol. Biol. **46:**225–236.
28. **Toleman, M. A., D. Biedenbach, D. Bennet, R. N. Jones, and T. R. Walsh.** 2003. Genetic characterization of a novel metallo beta-lactamase gene, $bla_{IMP\text{-}13}$, harboured by a novel Tn*5051*-type transposon disseminating carbapenemase genes in Europe: report from the SENTRY worldwide antimicrobial surveillance programme. J. Antimicrob. Chemother. **52:**583–590.
29. **Valverde, A., T. M. Coque, M. P. Sánchez-Moreno, A. Rollán, F. Baquero, and R. Cantón.** 2004. Dramatic increase in prevalence of fecal carriage of extended-spectrum β-lactamase-producing *Enterobacteriaceae* during nonoutbreak situations in Spain. J. Clin. Microbiol. **42:**4769–4775.
30. **Wachino, J., Y. Doi, K. Yamane, N. Shibata, T. Yagi, T. Kubota, and Y. Arakawa.** 2004. Molecular characterization of a cephamycin-hydrolyzing and inhibitor-resistant class A β-lactamase, GES-4, possessing a single G170S substitution in the Ω-loop. Antimicrob. Agents Chemother. **48:**2905–2910.
31. **Wang, H., S. Kelkar, W. Wu, M. Chen, and J. P. Quinn.** 2003. Clinical isolates of *Enterobacteriaceae* producing extended-spectrum β-lactamases: prevalence of CTX-M-3 at a hospital in China. Antimicrob. Agents Chemother. **47:**790–793.
32. **Yurieva, O., G. Kholodii, L. Minakhin, Z. Gorlenko, E. Kalyaeva, E. Mindlin, and V. Nikiforov.** 1997. Intercontinental spread of promiscuous mercury-resistance transposons in environmental bacteria. Mol. Microbiol. **24:**321–329.