Antimicrobial Agents and Chemotherapy

Novel Complex Class 1 Integron Bearing an IS *CR1* Element in an *Escherichia coli* Isolate Carrying the *bla*$_{CTX-M-14}$ Gene

Il Kwon Bae, You-Nae Lee, Wee Gyo Lee, Sang Hee Lee and Seok Hoon Jeong

Antimicrob. Agents Chemother. **2007, 51(8):3017. DOI: 10.1128/AAC.00279-07.**
Published Ahead of Print 21 May 2007.

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2007, p. 3017–3019 Vol. 51, No. 8
0066-4804/07/$08.00+0 doi:10.1128/AAC.00279-07

Novel Complex Class 1 Integron Bearing an IS*CR1* Element in an *Escherichia coli* Isolate Carrying the $bla_{CTX\text{-}M\text{-}14}$ Gene▽

Il Kwon Bae,[1] You-Nae Lee,[1] Wee Gyo Lee,[2] Sang Hee Lee,[3] and Seok Hoon Jeong[1]*

Research Institute for Antimicrobial Resistance and Department of Laboratory Medicine, Kosin University College of Medicine, 602-030, 34 Amnam-Dong, Suh-Gu, Busan,[1] Department of Laboratory Medicine, Ajou University School of Medicine, 442-749, San 5 Wonchun-Dong, Youngtong-Gu, Suwon,[2] and Department of Biological Sciences, Myongji University, 449-728, San 38-2 Nam-Dong, Yongin, Gyeonggido,[3] Korea

Received 24 February 2007/Returned for modification 20 April 2007/Accepted 8 May 2007

This work identifies an IS*CR1*-related $bla_{CTX\text{-}M\text{-}14}$ gene, which has never been reported before, from a clinical isolate of *Escherichia coli*. The $bla_{CTX\text{-}M\text{-}14}$ gene was preceded by an IS*CR1* element that was followed by a class 1 integron containing three different insert gene cassettes, i.e., *dfrA12*, *orfF*, and *aadA2*.

The CTX-M enzymes comprise more than 60 variants (http://www.lahey.org/studies/webt.asp) belonging to five different clusters (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25) on the basis of their amino acid sequence similarities (3). CTX-M enzymes have a wide substrate range, including penicillins and narrow- and expanded-spectrum cephalosporins, and as the designation CTX indicates, these enzymes preferentially hydrolyze cefotaxime but not ceftazidime, although some CTX-M enzymes, including CTX-M-15, CTX-M-19, and CTX-M-54 enzymes, have been associated with expansion of activity towards ceftazidime (2, 11, 13).

CTX-M-14 is a member of CTX-M-9 cluster and differs from CTX-M-9 only by the substitution Ala231Val (9). The amino acid sequences of CTX-M-14 and CTX-M-18 are identical (http://www.lahey.org/studies/webt.asp). CTX-M-14, one of the most widespread CTX-M enzymes, has been reported repeatedly in a very wide geographic area, including Europe, North America, South Asia, and East Asia (7, 10, 16). This enzyme has been found predominantly in *Enterobacteriaceae*, including *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella sonnei*, *Salmonella* spp., and *Proteus mirabilis* (7, 9, 15, 16, 19).

The rapid dissemination of CTX-M enzymes involves plasmid or strain epidemics, but it also involves mobile elements, including IS*Ecp1*-like insertion sequences and the IS*CR1* element (previously called *orf513*) (6). Most $bla_{CTX\text{-}M}$ genes belonging to the CTX-M-1, CTX-M-2, and CTX-M-9 clusters are associated with IS*Ecp1*-like insertion sequences, while IS*CR1* elements were identified upstream of the $bla_{CTX\text{-}M\text{-}2}$ and $bla_{CTX\text{-}M\text{-}9}$ genes (1, 6, 17). Interestingly, the $bla_{CTX\text{-}M\text{-}14}$ gene, although closely related to the $bla_{CTX\text{-}M\text{-}9}$ gene, has been reported to be mainly associated with IS*Ecp1* (6). This report identifies an IS*CR1*-related $bla_{CTX\text{-}M\text{-}14}$ gene, which has never been reported before, from a clinical isolate of *E. coli*.

E. coli AJE0508 was isolated from a sputum specimen from a 26-year-old male patient hospitalized at a tertiary care hospital in Suwon, Korea, in July 2005 for subarachnoid hemorrhage and pulmonary pneumonia. Strain AJE0508 exhibited resistance to ampicillin, ampicillin-sulbactam, ceftazidime, cefotaxime, aztreonam, tobramycin, and trimethoprim-sulfamethoxazole and susceptibility to cefoxitin, cefepime, imipenem, amikacin, gentamicin, and ciprofloxacin by disk diffusion assay. Agar dilution MIC testing on Mueller-Hinton agar (Difco Laboratories, Detroit, MI) with an inoculum of 10^4 CFU per spot confirmed that the strain was resistant to ampicillin (MIC, >256 μg/ml), ceftazidime (MIC, 32 μg/ml), cefotaxime (MIC, 64 μg/ml), and aztreonam (MIC, 64 μg/ml) but susceptible to cefoxitin (MIC, 8 μg/ml) and imipenem (MIC, 0.1 μg/ml) (5). Clavulanic acid (at a fixed concentration of 4 μg/ml) restored the activities of ceftazidime (MIC, 2 μg/ml) and cefotaxime (MIC, 1 μg/ml). The strain exhibited a positive double-disk synergy test, thus indicating the production of extended-spectrum beta-lactamases (16).

Plasmid analysis, which was carried with a commercial kit (QIAGEN, Valencia, CA) according to the instruction man-

TABLE 1. Primer sequences used in this study

PCR target	Primer name	Nucleotide sequence
5′CS element	5′CS-F	5′-CCAAGCTCTCGGGTAACATC-3′
dfrA12	dfrA12-F	5′-TTTATCTCGTTGCTGCGATG-3′
	dfrA12-R	5′-AGCTTGAATGGTTTCGGTTG-3′
aadA2	aadA2-F	5′-TCAGAGGTGCTAAGCGTCATT-3′
	aadA2-R	5′-GATCTCGCCTTTCACAAAGC-3′
qacEΔ1/sul1	3′CS-R	5′-GGGTTTCCGAGAAGGTGATT-3′
	Sul1-mF	5′-ACGAGATTGTGCGGTTCTTC-3′
IS*CR1*	ISCR1-F	5′-GCGAGTCAATCGCCCACT-3′
	ISCR1-mF	5′-GATGCCGAGAATACGTGGTT-3′
	ISCR1-R	5′-CGACTCTGTGATGGATCGAA-3′
	ISCR1-pR	5′-CAGACGCTCGTGATGACAAT-3′
IS*Ecp1*	TN1-F	5′-TCTGCTCCTTGAGAATGCAA-3′
	BTN1-F	5′-CGGTGGGTCATCTCTTGCTA-3′
$bla_{CTX\text{-}M\text{-}9}$ cluster	CTX-M-9-F	5′-TGCAACGGATGATGTTCG-3′
	CTX-M-9-mF	5′-ACGTGGCTCAAAGGCAATAC-3′
	CTX-M-9-R	5′-CGGCTGGGTAAAATAGGTCA-3′
	CTX-M-9-mR	5′-TCAATTTGTTCATGGCGGTA-3′
IS*903*-like element	IS903-R	5′-GGCATACCTGCTTTCGTCAT-3′

* Corresponding author. Mailing address: Department of Laboratory Medicine, Kosin University College of Medicine, 602-030, 34 Amnam-Dong, Suh-Gu, Busan, Republic of Korea. Phone: 82-51-990-6373. Fax: 82-51-990-3034. E-mail: kscpjsh@ns.kosinmed.or.kr.

▽ Published ahead of print on 21 May 2007.

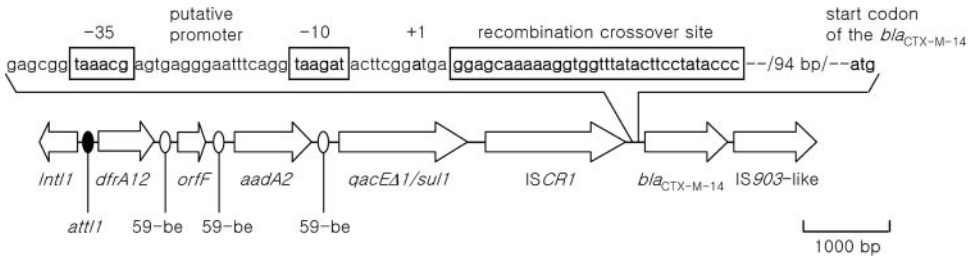

FIG. 1. Schematic map of the complex class 1 integron carrying the $bla_{CTX-M-14}$ gene on plasmid pAJE0508. Open arrows, open reading frames; white ovals, 59-be; black oval, *attI1* recombination site. The +1 transcription initiation site and the ATG start codon of the $bla_{CTX-M-14}$ gene are in boldface type. The −35 and −10 motifs of the putative promoter and the recombination crossover site are boxed.

ual, showed that *E. coli* AJE0508 contained two plasmids with molecular sizes of ca. 82 kbp (pAJE0508) and 25 kbp. The strain transferred pAJE0508 to the *E. coli* J53 azide-resistant recipient in mating experiments in which transconjugants were selected on Mueller-Hinton agar plates supplemented with cefotaxime (2 μg/ml) and sodium azide (100 μg/ml) (2). PCR amplifications using primers (16) specific for extended-spectrum beta-lactamase-encoding genes revealed that the transconjugant (*E. coli* trcAJE0508) possessed bla_{SHV}, bla_{TEM}, and $bla_{CTX-M-9}$ cluster genes. Sequences of the PCR amplicons for three type genes were 100% identical to the bla_{SHV-12}, bla_{TEM-1}, and $bla_{CTX-M-14}$ sequences, respectively. The bla_{SHV-12} and $bla_{CTX-M-14}$ genes might be attributed to high-level resistance of strain AJE0508 to cefotaxime and cetazidime.

The internal IS*CR1* (ISCR1-F and ISCR1-mF) or IS*Ecp1* (TN1-F and BTN1-F) forward primers and $bla_{CTX-M-9}$ cluster reverse primers (CTX-M-9-R and CTX-M-9-mR) were used to investigate the upstream region of the $bla_{CTX-M-14}$ gene. While a sequence having homology with the IS*CR1* element was detected upstream of the $bla_{CTX-M-14}$ gene on pAJE0508, IS*Ecp1*-like insertion sequences were not. These results suggested that the $bla_{CTX-M-14}$ gene is associated with a complex class 1 integron containing IS*CR1*, as the $bla_{CTX-M-9}$ gene on plasmid pMSP071 is associated with the complex In60 (17). To analyze the complex class 1 integron, sequencing of several overlapping PCR fragments obtained from pAJE0508 with primers corresponding to internal regions of In60 and the $bla_{CTX-M-14}$ gene was performed (Table 1).

The $bla_{CTX-M-14}$ gene was preceded by an IS*CR1* element that was followed by a typical class 1 integron containing two conserved elements, 5′-CS and 3′-CS (Fig. 1). The integron contained three different insert gene cassettes. The first contained a dihydrofolate reductase type A12 gene, *dfrA12* (previously called *dhfrXII*), which confers resistance to trimethoprim. The second contained an open reading frame, *orfF*, of unknown function, and the third contained an aminoglycoside adenyltransferase gene, *aadA2*, which confers resistance to streptomycin and spectinomycin (8). Each gene cassette contained a 59-base element recombination site. Although the *dfrA12-orfF-aadA2* array has been globally disseminated since it was first described in Finland in 1969 (8), this is the first report of the array being associated with the $bla_{CTX-M-14}$ gene.

An IS*CR1* element was found downstream of the 3′-CS element and upstream of the $bla_{CTX-M-14}$ gene. Recently it has been suggested that IS*CR* elements are members of an extended family of IS*91*-like elements that can transpose adjacent DNA sequences by a mechanism termed rolling-circle transposition and are responsible for the mobilization of virtually every class of antibiotic resistance genes, including the bla_{CTX-M} genes (18). A recombination crossover site (RCS) (33-bp DNA sequence) at which insertion of resistance genes into the complex class 1 integron containing IS*CR1* takes place (14) was observed within the 3′ noncoding sequence of IS*CR1*. A 94-bp region which is identical to that of In60 was identified between the RCS and the start codon of the $bla_{CTX-M-14}$ gene. A putative promoter consisting of the −35 (TAAACG) and −10 (TAAGAT) regions, which drives $bla_{CTX-M-14}$ transcription, was observed just upstream of the RCS. The −35 and −10 sequences were separated by 17 bp.

Sequence analysis of the downstream DNA of the $bla_{CTX-M-14}$ gene in the complex class 1 integron on the pAJE0508 revealed the presence of an IS*903*-like element, the role of which in the mobilization process of the bla_{CTX-M} genes has not yet been demonstrated (12). This insertion sequence has been found downstream of the $bla_{CTX-M-17}$, $bla_{CTX-M-19}$, $bla_{CTX-M-24}$, and $bla_{CTX-M-54}$ genes (2, 4, 6, 12) and also has been identified downstream of the $bla_{CTX-M-14}$ genes in three *E. coli* strains and one *K. pneumoniae* strain recently isolated in Paris (6).

This work describes a complex class 1 integron bearing an IS*CR1* element and shows that mobilization and expression of the $bla_{CTX-M-14}$ gene may be associated with IS*CR1* elements. The IS*CR1* element is a powerful genetic element that can mobilize antibiotic resistance genes, so further spread of the $bla_{CTX-M-14}$ gene can be anticipated.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper are available in the GenBank nucleotide database under accession number EF450247.

This work was supported by a Korea Research Foundation grant (KRF-2006-331-E00455).

REFERENCES

1. **Arduino, S. M., P. H. Roy, G. A. Jacoby, B. E. Orman, S. A. Pineiro, and D. Centron.** 2002. $bla_{CTX-M-2}$ is located in an unusual class 1 integron (In35) which includes Orf513. Antimicrob. Agents Chemother. **46:**2303–2306.
2. **Bae, I. K., B. H. Lee, H. Y. Hwang, S. H. Jeong, S. G. Hong, C. L. Chang, H. S. Kwak, H. J. Kim, and H. Youn.** 2006. A novel ceftazidime-hydrolysing extended-spectrum beta-lactamase, CTX-M-54, with a single amino acid substitution at position 167 in the omega loop. J. Antimicrob. Chemother. **58:**315–319.
3. **Bonnet, R.** 2004. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother. **48:**1–14.
4. **Cao, V., T. Lambert, and P. Courvalin.** 2002. ColE1-like plasmid pIP843 of

Klebsiella pneumoniae encoding extended-spectrum β-lactamase CTX-M-17. Antimicrob. Agents Chemother. **46:**1212–1217.
5. **Clinical and Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed., M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA.
6. **Eckert, C., V. Gautier, and G. Arlet.** 2006. DNA sequence analysis of the genetic environment of bla_{CTX-M} genes. J. Antimicrob. Chemother. **57:**14–23.
7. **Eckert, C., V. Gautier, M. Saladin-Allard, N. Hidri, C. Verdet, Z. Ould-Hocine, G. Barnaud, F. Delisle, A. Rossier, T. Lambert, A. Philippon, and G. Arlet.** 2004. Dissemination of CTX-M-type beta-lactamases among clinical isolates of *Enterobacteriaceae* in Paris, France. Antimicrob. Agents Chemother. **48:**1249–1255.
8. **Gestal, A. M., H. W. Stokes, S. R. Partridge, and R. M. Hall.** 2005. Recombination between the *dfrA12-orfF-aadA2* cassette array and an *aadA1* gene cassette creates a hybrid cassette, *aadA8b*. Antimicrob. Agents Chemother. **49:**4771–4774.
9. **Pai, H., E. H. Choi, H. J. Lee, J. Y. Hong, and G. A. Jacoby.** 2001. Identification of CTX-M-14 extended-spectrum beta-lactamase in clinical isolates of *Shigella sonnei*, *Escherichia coli*, and *Klebsiella pneumoniae* in Korea. J. Clin. Microbiol. **39:**3747–3749.
10. **Pitout, J. D., D. B. Gregson, D. L. Church, S. Elsayed, and K. B. Laupland.** 2005. Community-wide outbreaks of clonally related CTX-M-14 beta-lactamase-producing *Escherichia coli* strains in the Calgary health region. J. Clin. Microbiol. **43:**2844–2849.
11. **Poirel, L., M. Gniadkowski, and P. Nordmann.** 2002. Biochemical analysis of the ceftazidime-hydrolysing extended-spectrum beta-lactamase CTX-M-15 and of its structurally related beta-lactamase CTX-M-3. J. Antimicrob. Chemother. **50:**1031–1034.
12. **Poirel, L., M. F. Lartigue, J. W. Decousser, and P. Nordmann.** 2005. IS*Ecp1B*-mediated transposition of bla_{CTX-M} in *Escherichia coli*. Antimicrob. Agents Chemother. **49:**447–450.
13. **Poirel, L., T. Nass, I. Le Thomas, A. Karim, E. Bingen, and P. Nordmann.** 2001. CTX-M-type extended-spectrum beta-lactamase that hydrolyzes ceftazidime through a single amino acid substitution in the omega loop. Antimicrob. Agents Chemother. **45:**3355–3361.
14. **Rodriguez-Martinez, J. M., L. Poirel, R. Canton, and P. Nordmann.** 2006. Common region CR1 for expression of antibiotic resistance genes. Antimicrob. Agents Chemother. **50:**2544–2546.
15. **Romero, L., L. Lopez, L. Martinez-Martinez, B. Guerra, J. R. Hernandez, and A. Pascual.** 2004. Characterization of the first CTX-M-14-producing *Salmonella enterica* serotype Enteritidis isolate. J. Antimicrob. Chemother. **53:**1113–1114.
16. **Ryoo, N. H., E. C. Kim, S. G. Hong, Y. J. Park, K. Lee, I. K. Bae, E. H. Song, and S. H. Jeong.** 2005. Dissemination of SHV-12 and CTX-M-type extended-spectrum β-lactamases among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* and emergence of GES-3 in Korea. J. Antimicrob. Chemother. **56:**698–702.
17. **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 $bla_{CTX-M-9}$. Antimicrob. Agents Chemother. **46:**2656–2661.
18. **Toleman, M. A., P. M. Bennett, and T. R. Walsh.** 2006. IS*CR* elements: novel gene-capturing systems of the 21st century? Microbiol. Mol. Biol. Rev. **70:** 296–316.
19. **Wu, L. T., H. J. Wu, J. G. Chung, Y. C. Chuang, K. C. Cheng, and W. L. Yu.** 2006. Dissemination of *Proteus mirabilis* isolates harboring CTX-M-14 and CTX-M-3 beta-lactamases at 2 hospitals in Taiwan. Diagn. Microbiol. Infect. Dis. **54:**89–94.