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NOTE

Nomenclature of IS*CR1* Elements Capable of Mobilizing Antibiotic Resistance Genes Present in Complex Class 1 Integrons

Seung Ghyu Sohn[1†], Jae Jin Lee[1†], Jae Seok Song[1], Jung Hun Lee[1,3], Ha Ik Sun[1], Kwang Seung Park[1], Il Kwon Bae[2], Jung-Hyun Lee[3], Byeong Chul Jeong[1], and Sang Hee Lee[1*]

[1]*Drug Resistance Proteomics Laboratory, Department of Biological Sciences, Myongji University, Gyeonggido 449-728, Republic of Korea*
[2]*Research Institute for Antimicrobial Resistance, Kosin University College of Medicine, Busan 602-030, Republic of Korea*
[3]*Marine Biotechnology Research Center, Korea Ocean Research and Development Institute, Ansan 425-600, Republic of Korea*

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The dissemination of many antibiotic resistance genes has arisen among members of the family *Enterobacteriaceae*. The dissemination mechanism of these antibiotic resistance genes is closely linked with insertion sequence common region 1 (IS*CR1*). Thus, caution must be taken in clinical settings to prevent further dissemination of these antibiotic resistance genes. A nomenclature system of IS*CR1* variants, important for the antibiotic resistance dissemination, was proposed. The proposed system can designate all IS*CR1* variants on the basis of the detection time and by considering amino-acid substitution(s) compared with IS*CR1a*. This nomenclature system of IS*CR1* variants can be applied to 19 groups (IS*CR1* to IS*CR19*) of the IS*CR* family and help some researchers to correctly designate new IS*CR* subgroups.

***Keywords*:** nomenclature, IS*CR1*, complex class 1 integron

Severe clinical problems arise from the emergence and dissemination of antibiotic resistance in pathogens causing nosocomial infections (Lee *et al*., 2009). The dissemination of *dfrA10*, *bla*$_{CMY}$, *bla*$_{MOX-1}$, *bla*$_{DHA-1}$, *bla*$_{CTX-M}$, *bla*$_{VEB-3}$, *bla*$_{PER-1}$, *catA2*, and *qnr* genes has arisen among members of the family *Enterobacteriaceae* (Ambler, 1980; Stokes *et al*., 1993; Verdet *et al*., 2000; Doi *et al*., 2002; Sorum *et al*., 2003; Jeong *et al*., 2005; Lee *et al*., 2009). A new type of genetic element (IS*CR*: insertion sequence common region) has been identified as being closely linked with the dissemination of these antibiotic resistance genes (Toleman *et al*., 2006a, 2006b; Walsh, 2006). Recently, it has been suggested that IS*CR1* element is a member 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 many classes of antibiotic resistance genes, including *dfrA10*, *bla*$_{CMY}$, *bla*$_{MOX-1}$, *bla*$_{DHA-1}$, *bla*$_{CTX-M}$, *bla*$_{VEB-3}$, *bla*$_{PER-1}$, *catA2*, and *qnr* genes located in complex class 1 integrons (Toleman *et al*., 2006a, 2006b). A recombination crossover site (RCS) (33 bp DNA sequence containing *ori*IS) at which insertion of resistance genes into the complex class 1 integron containing IS*CR1* takes place was observed downstream of IS*CR1* (Bennett, 2008). A 179 bp region, which was identified between the RCS and the start codon of the *bla*$_{CMY-10}$ gene, has not been identified to any known nucleotide sequences (Lee *et al*., 2003, 2006). The unique region has been first identified in the integron (GenBank accession no. FJ004895) harboring the *bla*$_{CMY-10}$ gene. Furthermore, the genetic environment surrounding the 3' end of RCS revealed that the upstream sequence of *bla*$_{CMY-10}$ had an additional 15 bases compared with the upstream sequence of *bla*$_{CMY-1}$ (Bauernfeind *et al*., 1996). On the other hand, the upstream sequences of *bla*$_{CMY-9}$ (Doi *et al*., 2002) and *bla*$_{CMY-8}$ (Chen *et al*., 2007) lacked 50 bases compared with the upstream sequence of *bla*$_{CMY-1}$. The nucleotide sequence analysis of *bla*$_{CMY-1}$, *bla*$_{CMY-8}$, *bla*$_{CMY-9}$, and *bla*$_{CMY-10}$ genes made in his study indicates that there might be past transposition events by the IS*CR1* element upstream of *bla*$_{CMY}$ genes because of different genetic environments surrounding the 3' end of RCS in these genes. A previous report suggested that this variation is indicative of past transposition events (Doi *et al*., 2002).

According to sequence identity comparisons of tranposases encoded by IS*CR* elements, there are 19 groups (IS*CR1* to IS*CR19*) of the IS*CR* family at present (http://www.cardiff.ac.uk/medic/aboutus/departments/medicalmicrobiology/genetics/iscr/iscr_elements.html) (Toleman and Walsh, 2008). To date, two subgroups of IS*CR5* were designated as IS*CR5a* and ISC*R5b*. Compared with the deduced amino-acid sequence of IS*CR5a*, ISC*R5b* showed eight amino-acid differences. However, this nomenclature system is temporary and is not systematic (unified). Furthermore, more than 20 IS*CR1* genes have been reported in Australia, Argentina, Italy, and Re-

† These authors contributed equally to this work.
∗ To whom correspondence should be addressed.
(Tel) 82-31-330-6195; (Fax) 82-31-335-8249
(E-mail) sangheelee@mju.ac.kr

Table 1. Nomenclature, epidemiology, and amino-acid differences of the IS*CR1* subgroups

Designation[b]	GenBank accession number (plasmid)	Country of origin	Clinical isolates	Amino-acid (coding triplet) differences[a]											
				15	112	117	187	292	329	383	384	393	407	426	452
IS*CR1a*[c]	L06822 (pSa)	Australia	*Escherichia coli*	Gln (CAA)	Ile (ATA)	Pro (CCC)	Asn (AAT)	Glu (GAG)	Tyr (TAC)	Val (GTC)	Asn (AAC)	Ala (GCA)	Arg (CGG)	Pro (CCT)	His (CAC)
IS*CR1b*$_1$	AJ311891 (pS21)	Argentina	*Salmonella enterica*-Infantis									Val (GTA)			
IS*CR1b*$_2$	AJ746361 (t-ST4)	Italy	*S. enterica*-Typhimurium					Lys (AAG)							
IS*CR1b*$_3$	AY878718 (pKO56) AY878717 (pKO97)	South Korea	*E. coli*												Pro (CCC)
IS*CR1e*	FJ004895 (pYMG-5)	South Korea	*Enterobacter aerogenes*		Val (GTG)	Leu (CTC)	Ser (AGT)		His (CAC)						
IS*CR1f*	AJ310778 (incF1)	Italy	*S. enterica*-Typhimurium	Arg (CGA)						Ala (GCA)	Tyr (TAC)		Gln (CAG)	Leu (CTT)	

[a] Amino-acid residue and coding triplet differences are depicted in relation to the coding sequence of IS*CR1a* (L06822).
[b] IS*CR1a* is named for IS*CR1* (L06822) first detected in a clinical isolate. IS*CR1b* (IS*CR1e* or IS*CR1f*) is named for IS*CR1* with one (four or five) amino-acid difference(s) compared with IS*CR1a*. IS*CR1c* (or IS*CR1d*) with two (or three) amino-acid differences has not been detected. IS*CR1b*$_1$ (IS*CR1b*$_2$ or IS*CR1b*$_3$) is named for the subgroup of IS*CR1b* in which the amino-acid change position was detected for the first (second or third).
[c] Other locations [GenBank accession number (plasmid, chromosome, or integron)] of IS*CR1a* (Toleman *et al*., 2006a; Chen *et al*., 2007): L06418 (pDGO10), EF450247 (pAJE0508), AY740681 (In39), AF174129 (pMSP071), AY079169 (pMAR12), AB061794 (pCMXR1), AF550415 (pCTX-M3), AY259085 (pHSH1), AY259086 (pHSH2), AY049746 (chromosome), AY123253 (pRMH760), AY162283 (In38), AJ517791 (pAr-32), and EF382672 (pK29).

public of Korea (Table 1). According to our analyses of their deduced amino-acid sequences, four subgroups (variants showing four different types of missense mutations) of IS*CR1* were detected in clinical isolates. Therefore, we propose a nomenclature system of IS*CR1* variants in Table 1.

The BLASTN (Basic Local Alignment Search Tool against Nucleotide sequence database) program of NCBI (National Center for Biotechnology Information: http://www.ncbi.nlm.nih.gov) was used for database searches of IS*CR1* genes, and the CLUSTAL W program (Thompson *et al*., 1994) was used to align multiple nucleotide and deduced protein sequences. Nucleotide sequence accession numbers were taken from GenBank (Genetic Sequence Database, USA), EMBL (European Molecular Biology Laboratory, Germany), and/or DDBJ (DNA Data Bank of Japan, Japan) databases. DNA sequence analysis was performed with DNASIS for Windows (Hitachi Software Engineering America Ltd., USA).

In the nomenclature system of IS*CR1* variants in Table 1, IS*CR1a* is named for IS*CR1* (GenBank accession no. L06822) (Stokes *et al*., 1993) first detected in a clinical isolate. IS*CR1b* (IS*CR1e* or IS*CR1f*) is named for IS*CR1* with one (four or five) amino-acid substitution(s) compared with IS*CR1a*. IS*CR1c* (or IS*CR1d*) with two (or three) amino-acid differences has not been detected in clinical isolates. IS*CR1b*$_1$ (IS*CR1b*$_2$ or IS*CR1b*$_3$) is named for the subgroup of IS*CR1b* in which the amino-acid change position was detected for the first (second or third). IS*CR1a*, identified in 78% (15 of 21) IS*CR1* variants, is the predominant group detected among enterobacterial isolates (Table 1). IS*CR1b*$_1$, IS*CR1b*$_2$, and IS*CR1b*$_3$ had one amino-acid substitution (Ala 393Val, Glu292Lys, and His452Pro, respectively). IS*CR1e* showed Ile112Val, Pro117Leu, Asn187Ser, and Tyr329His substitutions. IS*CR1f* had five amino-acid substitutions (Gln 15Arg, Val383Ala, Asn384Tyr, Arg407Gln, and Pro426Leu). It is not known how each amino-acid substitution in IS*CR1* variants appears to have changes in their crystal structures and then affects efficiencies of their transposition. To elucidate this molecular basis of amino-acid substitution(s), the structural analysis of IS*CR1* gene product will be performed.

This nomenclature system of IS*CR1* variants can be applied to other 18 groups (IS*CR2* to IS*CR19*) of the IS*CR* family and help some researchers to correctly designate new IS*CR* subgroups. As an example, the previously described IS*CR5b* can be renamed as IS*CR5i* because of its eight amino-acid substitutions. Because gene products of IS*CR1* variants are more than 95% identical to each other (Toleman *et al*., 2006b), more than 5% (>25 of 513 amino acids in an IS*CR1* gene product) amino-acid differences in a newly identified IS*CR*, compared with IS*CR1*, mean that the identified IS*CR* is a new group (e.g., IS*CR20*, and so on) of IS*CR* and not a new subgroup of IS*CR1*. Thus, alphabets can be used to classify all IS*CR1* variants into 25 subgroups, IS*CR1a* to IS*CR1y*. The disadvantage and advantage of this proposed system are as follows. First, IS*CR1* variants, designated on the basis of the traditional nomenclature system depending on the detection time, have to be renamed according to the proposed system. However, none of the IS*CR1* variants that are important for the dissemination of many antibiotic resistance genes have been designated on the basis of the traditional nomenclature system to date. Therefore, the proposed

system can be used to designate all IS*CR1* variants without being renamed. Second, the proposed system cannot name IS*CR1* variants in sequential order and can miss some names of IS*CR1* variants (e.g., IS*CR1c* and IS*CR1d* in Table 1) because they have not been detected in clinical isolates. However, if IS*CR1* variants are to be named only in sequential order without some missing points and without considering amino-acid substitution(s) compared with IS*CR1a*, alphabets cannot be used to classify all IS*CR1* variants because there are many (more than 25) different subgroups based on the detection time. Thus, the proposed system can designate all IS*CR1* variants (e.g., IS$CR1b_1$, IS$CR1b_2$, IS$CR1b_3$, and so on) on the basis of the detection time and by considering amino-acid substitution(s).

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