Molecular Characterization of IS*CR1*-Mediated *bla*$_{PER-1}$ in a Non-O1, Non-O139 *Vibrio cholerae* Strain from China

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We report the detection of PER-1 extended-spectrum β-lactamase (ESBL) in a clinical non-O1, non-O139 *Vibrio cholerae* strain from China. IS*CR1*-mediated *bla*$_{PER-1}$ was embedded in a complex In*4* family class 1 integron belonging to the lineage of Tn*1696* on a conjugative IncA/C plasmid. A free 8.98-kb circular molecule present with the IS*CR1*-*bla*$_{PER-1}$–truncated 3′-conserved sequence (CS) structure was detected in this isolate. These findings may provide insight into the mobilization of *bla*$_{PER-1}$.

Extended-spectrum β-lactamases (ESBLs) are uncommon in *Vibrio cholerae*. However, CTX-M-type, PER-2, and TEM-63 enzymes have been identified in serogroup O1 isolates from Argentina and South Africa (1, 2). PER β-lactamases belong to the ESBL family of enzymes, of which 7 variants have been identified to date. PER-1 was mainly detected in Europe, particularly in Turkey, and Asia (3, 4); PER-2 (86% amino acid sequence identity with PER-1) was reported in South America, mainly in Argentina (5, 6); PER-3, -4, and -5 (all differing by 1 amino acid from PER-1) were reported only in the GenBank database (accession numbers AY740681, EU748544, and EU687473, respectively); PER-6 (92% amino acid sequence identity with PER-2) and PER-7 (differing from PER-1 by 4 amino acids) were identified in Paris, France (7, 8).

In 2005, a non-O1, non-O139 *V. cholerae* strain, RJ354, was isolated from the blood sample of a hemodialysis patient in the Ruijin Hospital, Shanghai Jiaotong University School of Medicine. In this strain, two novel integron-borne cassettes, *dfrA27* and *aadA16*, were found to be located on a conjugative plasmid in our previous study (9). Initial susceptibility testing via disk diffusion revealed a high level of resistance to ceftazidime in RJ354. The MICs for ceftazidime, cefotaxime, piperacillin-tazobactam, and ceftazidime-clavulanic acid for RJ354, determined by the Etest method (AB bioMérieux, Askim, Sweden), were >256, 256, 2, and 0.25 mg/liter, respectively, indicating the presence of an ESBL. A conjugation experiment using *Escherichia coli* J53Az^r (sodium azide resistant) as the recipient demonstrated that resistance to ceftazidime can be transferred from RJ354 to J53Az^r. The susceptibilities of the transconjugant to ceftazidime (MIC, >256 mg/liter), cefotaxime (MIC, 128 mg/liter), piperacillin-tazobactam (MIC, 1 mg/liter), and ceftazidime-clavulanic (MIC, 0.19 mg/liter) were very similar to those of the donor, RJ354.

ESBL genes (*bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{PER}$, and *bla*$_{VEB}$) from strain RJ354 and its transconjugant were screened by PCR (10). Sequencing analysis of *bla*$_{PER}$-positive PCR products revealed the presence of PER-1 ESBL in both strains. The plasmid DNA content of isolate RJ354 and its transconjugant was examined using pulsed-field gel electrophoresis (PFGE) with S1 nuclease digestion, as described previously (11). The location of *bla*$_{PER-1}$ was determined by blotting the S1 nuclease-linearized PFGE-separated plasmid DNA onto positively charged nylon membranes (Roche Applied Science, Penzberg, Germany), with a digoxigenin-labeled *bla*$_{PER-1}$-specific probe. Plasmid incompatibility

groups were determined by a PCR-based replicon typing scheme described by Carattoli et al. (12). The results revealed that *bla*$_{PER-1}$ was located on a 160-kb broad-host-range IncA/C plasmid within the RJ354 isolate and its transconjugant. In previous studies, *bla*$_{PER-1}$ was also found to be located on IncA/C plasmids in *Providencia stuartii* and *Klebsiella pneumoniae* strains from Tunisia and South Korea, respectively (10, 13), indicating that the IncA/C plasmids may play a role in the dissemination of *bla*$_{PER-1}$.

Overlap PCR was performed to determine the genetic context of *bla*$_{PER-1}$; primers specific for sequences that typically surround *bla*$_{PER-1}$ were used in the PCR experiment (Table 1). PCR amplification and sequencing of the region located upstream of *bla*$_{PER-1}$ revealed an IS*CR1* element. The spacer between IS*CR1* and *bla*$_{PER-1}$ was 62 bp long and was identical to the spacer detected in the previously described isolate (14–17). The association of IS*CR1* with *bla*$_{PER-1}$ was first identified in an *Aeromonas punctata* strain isolated from China in 2008 (15), 3 years after the isolation of the non-O1, non-O139 *V. cholerae* strain in the present study. To date, all *bla*$_{PER-1}$ genes reported in strains from China were preceded by an IS*CR1* element (14–17), while in strains from outside China, the insertion sequence IS*Pa12* was always located upstream of *bla*$_{PER-1}$ (4), suggesting that there may be two divergent paths in the evolution of *bla*$_{PER-1}$. Because IS*CR1* was identified in the Chinese isolates, PCR primers (P3, P2) located within the 5′-conserved sequence (CS) of class 1 integron and *bla*$_{PER-1}$ were used to investigate the sequences further upstream of *bla*$_{PER-1}$. As expected, a typical class 1 integron containing an array of *arr-3*–*dfrA27*–*aadA16* gene cassettes was detected; this array was described previously in the same strain in our laboratory (9). Different Chinese isolates harboring IS*CR1*-*bla*$_{PER-1}$ have distinct

TABLE 1 Primers used in this study

Primer	Target gene or region	Primer sequence (5′ to 3′)	PCR product size (kb)
P1	IS*CR1*	GATACTAACTGGCGTGACAAGAG	0.97
P2	bla_{PER-1}	CTCGTCTCCCTGATACGCTTTC	
P3	*IntI*	CGAACCCAGTGGACATAAGCC	6.3
P2	bla_{PER-1}	CTCGTCTCCCTGATACGCTTTC	
P4	*tnpR* of Tn*1696*	GCCCTTCTTTGACGAACTCCA	7.8
P2	bla_{PER-1}	CTCGTCTCCCTGATACGCTTTC	
P5	bla_{PER-1}	CGTATCAGGGAGACGAGTTTAGT	4.6
P6	Downstream of *abct*	GGTAGCGAGATTCACAGACAGA	
P7	*abct*	CCACCACATACACCATCACATCC	4.0
P8	*orf5*	CATCAGCCGCACAACCTCGTC	
P7	*abct*	CCACCACATACACCATCACATCC	5.7
P9	IS*6100*	AGGCGGCTGCTGCGAAATGGTG	
P2	bla_{PER-1}	CTCGTCTCCCTGATACGCTTTC	6.4
P7	*abct*	CCACCACATACACCATCACATCC	

arrays of gene cassettes upstream of IS*CR1* (Fig. 1) (14, 15), suggesting that different recombination events were responsible for the IS*CR1*-mediated integration of bla_{PER-1} into class 1 integrons.

Sequence analysis of the region downstream of bla_{PER-1} demonstrated the presence of an array of bla_{PER-1}-*gst* (encoding a glutathione *S*-transferase)-*abct* (encoding an ABC-type transporter) identical to the corresponding region described in the *Acinetobacter johnsonii* XBB1 strain from China in 2014 (14), followed by *qacEΔ1/sul1* and *orf5*, which formed the 3′ end of the novel complex class 1 integron identified in the present study. Notably, at the start of the second copy of 3′ CS (3′ CS2, *qacEΔ1/sul1*), a 179-bp

deletion was observed, in comparison with the full-length copy of 3′ CS1.

In *A. johnsonii* XBB1, the complex class 1 integron harboring the IS*CR1*-bla_{PER-1} structure was flanked by the same miniature inverted-repeat transposable element (MITE) (Fig. 1) (14). However, in the present study, this MITE structure was not detected by PCR. Subsequently, two pairs of primers (P2/P4 and P7/P9) were designed on the basis of the previously reported gene structure of transposon Tn*1696* (18), and two unique amplicons of 7.8 kb and 5.7 kb were obtained and sequenced. Analysis of the whole 16,020-bp fragment revealed that the complex class 1 integron was preceded by the *tnpR* of Tn*1696* at the 5′ end and was followed by an IS*6100* insertion sequence at the 3′ end (Fig. 1). These findings indicated that IS*CR1*-mediated bla_{PER-1} was embedded in a novel complex In*4* family class 1 integron belonging to the lineage of Tn*1696*.

The IS*CR1* element is an unusual insertion sequence that demonstrates IS*91*-like characteristics and may mobilize adjacent DNA sequences via a process called rolling-circle replication (19). Partridge and Hall (20) confirmed that a small circular molecule containing IS*CR1*-*dfrA10*-*sul1* generated by restriction digestion of the complex class 1 integron In*34* can be transposed into a plasmid at a site within the 3′ CS of a cloned class 1 integron. To investigate whether such a circular molecule was present in the RJ354 strain, PCR amplification was performed with the inverse primers (P2, P7) located within bla_{PER-1} and *abct* (Fig. 1), and an amplicon of 6.4 kb was obtained, suggesting the presence of a circular molecule. Sequence analysis of the complete 8,980-bp circular molecule was performed to elucidate the structure of IS*CR1*-bla_{PER-1}-*gst*-*abct*-3′ CS (with a 179-bp deletion at the beginning of *qacEΔ1*); the deduced structure was identical to that of the
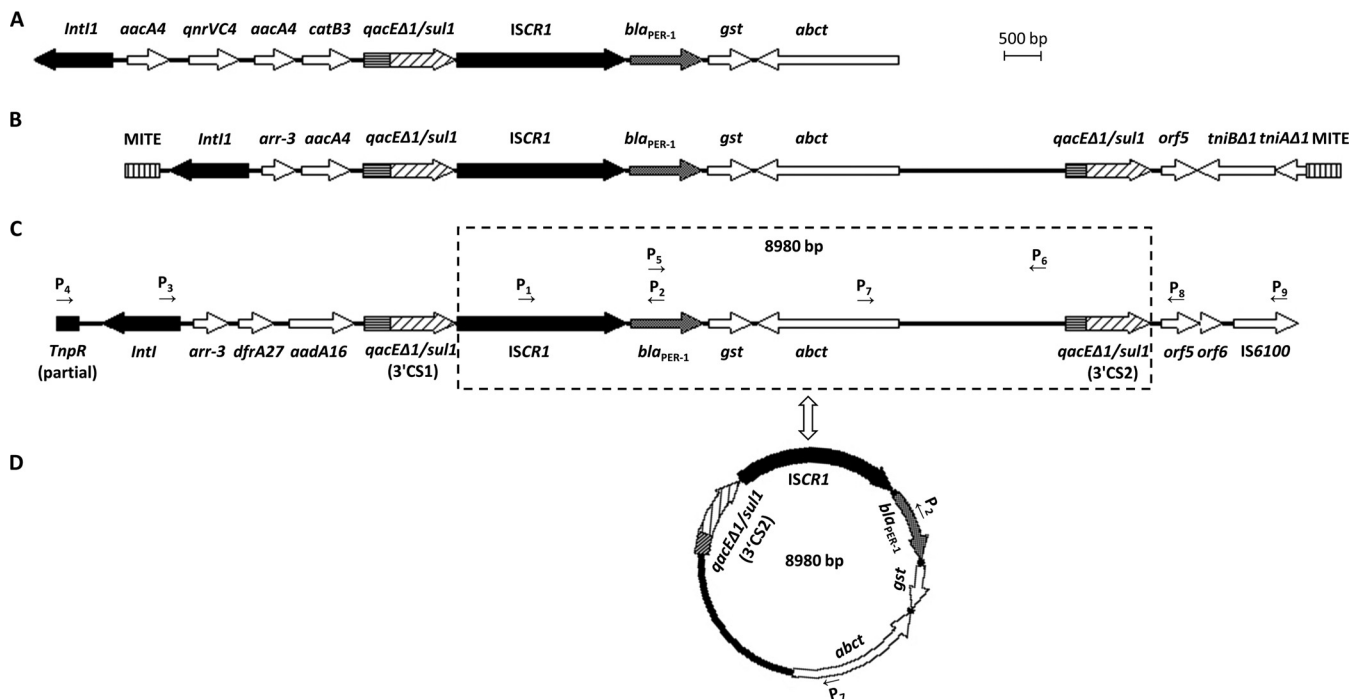

FIG 1 Genetic environment of IS*CR1*-mediated bla_{PER-1} in *Acinetobacter johnsonii* XBB1 (GenBank accession no. KF017283) (A), *Aeromonas punctate* 159 (GenBank accession no. GQ891757) (B), and non-O1, non-O139 *Vibrio cholerae* of this study (C). Open arrows indicate genetic orientations; thin horizontal arrows indicate primers (P); and the dotted box shows the fragment corresponding to panel D. (D) Small circular molecule detected in this study.

corresponding region in the complex class 1 integron identified in the present study (Fig. 1). Therefore, it is likely that the circular molecule was formed by excision and circularization of the corresponding region of the complex class 1 integron and vice versa. These findings strongly suggest that IS*CR1*-bla_{PER-1}-truncated 3′ CS may function as a powerful genetic vehicle that can facilitate horizontal dissemination of bla_{PER-1}.

In conclusion, our study describes, for the first time, the identification of a PER-1 ESBL in a non-O1, non-O139 *V. cholerae* strain. bla_{PER-1} is carried on a conjugative and broad-host-range IncA/C plasmid; a Tn*1696*-like transposon is located in this plasmid, and a highly mobile IS*CR1* element is embedded in this transposon and is present in the form of a small circular molecule as well.

Nucleotide sequence accession number. The sequence of the genetic environment of bla_{PER-1} in non-O1, non-O139 *V. cholerae* RJ354 has been deposited in GenBank under the accession number KP076293.

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