Molecular Characterization of ISCR1-Mediated bla<sub>PER-1</sub> in a Non-O1, Non-O139 Vibrio cholerae Strain from China

Jun Wu,a Lianyan Xie,b Fangfang Zhang,b Yuxing Nib and Jingyong Sunb

Department of Critical Care Medicine, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China; aDepartment of Clinical Microbiology, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

We report the detection of PER-1 extended-spectrum β-lactamase (ESBL) in a clinical non-O1, non-O139 Vibrio cholerae strain from China. ISCR1-mediated bla<sub>PER-1</sub> was embedded in a complex IncF family class 1 integron belonging to the lineage of Tn1696 on a conjugative IncA/C plasmid. A free 8.98-kb circular molecule present with the ISCR1-bla<sub>PER-1</sub>-truncated 3′-conserved sequence (CS) structure was detected in this isolate. These findings may provide insight into the mobilization of bla<sub>PER-1</sub>.

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 conjugal 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 cefotaxime-clavulanic acid for RJ354, determined by the E-test method (AB bioMérieux, Askim, Sweden), were >256, 256, 2, and 0.25 mg/liter, respectively, indicating the presence of an ESBL. A previous study (9) revealed a high level of resistance to ceftazidime in RJ354. The susceptibility testing also demonstrated that resistance to ceftazidime can be transferred from RJ354 to J53Azr. The recipient demonstrated that resistance to ceftazidime can be transferred from RJ354 to J53Azr. 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<sub>TEM</sub>, bla<sub>CTX-M</sub>, bla<sub>SHV</sub>, bla<sub>PER</sub>, and bla<sub>VER</sub>) from strain RJ354 and its transconjugant were screened by PCR (10). Sequencing analysis of bla<sub>PER</sub>-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<sub>PER</sub>-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<sub>PER</sub>-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<sub>PER</sub>-1 was located on a 160-kb broad-host-range IncA/C plasmid within the RJ354 isolate and its transconjugant. In previous studies, bla<sub>PER</sub>-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<sub>PER</sub>-1.

Overlap PCR was performed to determine the genetic context of bla<sub>PER</sub>-1; primers specific for sequences that typically surround bla<sub>PER</sub>-1 were used in the PCR experiment (Table 1). PCR amplification and sequencing of the region located upstream of bla<sub>PER</sub>-1 revealed an ISCR1 element. The spacer between ISCR1 and bla<sub>PER</sub>-1 was 62 bp long and was identical to the spacer detected in the previously described isolate (14–17). The association of ISCR1 with bla<sub>PER</sub>-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<sub>PER</sub>-1 genes reported in strains from China were preceded by an ISCR1 element (14–17), while in strains from outside China, the insertion sequence ISPa12 was always located upstream of bla<sub>PER</sub>-1 (4), suggesting that there may be two divergent paths in the evolution of bla<sub>PER</sub>-1. Because ISCR1 was identified in the Chinese isolates, PCR primers (P3, P2) located within the 5′-conserved sequence (CS) of class 1 integron and bla<sub>PER</sub>-1 were used to investigate the sequences further upstream of bla<sub>PER</sub>-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 ISCR1-bla<sub>PER</sub>-1 have distinct
arrays of gene cassettes upstream of IS\textsubscript{CR1} (Fig. 1) (14, 15), suggesting that different recombination events were responsible for the IS\textsubscript{CR1}-mediated integration of \textit{bla\textsubscript{PER-1}} into class 1 integrons.

Sequence analysis of the region downstream of \textit{bla\textsubscript{PER-1}} demonstrated the presence of an array of \textit{bla\textsubscript{PER-1}}-\textit{gst} (encoding a glutathione S-transferase)-\textit{abct} (encoding an ABC-type transporter) identical to the corresponding region described in the \textit{Acinetobacter johnsonii} XBB1 strain from China in 2014 (14), followed by \textit{qacE\textsubscript{1}}/\textit{H9004}/\textit{sul1} and \textit{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, \textit{qacE\textsubscript{1}}/\textit{sul1}), a 179-bp deletion was observed, in comparison with the full-length copy of 3' CS1.

In \textit{A. johnsonii} XBB1, the complex class 1 integron harboring the IS\textsubscript{CR1}-\textit{bla\textsubscript{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 \textit{Tn1696} (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 \textit{tnpR} of \textit{Tn1696} at the 5' end and was followed by an IS\textsubscript{6100} insertion sequence at the 3' end (Fig. 1). These findings indicated that IS\textsubscript{CR1}-mediated \textit{bla\textsubscript{PER-1}} was embedded in a novel complex \textit{In4} family class 1 integron belonging to the lineage of \textit{Tn1696}.

The IS\textsubscript{CR1} element is an unusual insertion sequence that demonstrates IS91-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\textsubscript{CR1}-\textit{dfrA10}-\textit{sul1} generated by restriction digestion of the complex class 1 integron \textit{In34} 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 \textit{bla\textsubscript{PER-1}} and \textit{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\textsubscript{CR1}-\textit{bla\textsubscript{PER-1}}-\textit{gst}-\textit{abct}-3' CS2 (with a 179-bp deletion at the beginning of \textit{qacE\textsubscript{1}}); the deduced structure was identical to that of the

![TABLE 1](https://example.com/table1.png)

**TABLE 1** Primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Target gene or region</th>
<th>Primer sequence (5' to 3')</th>
<th>PCR product size (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>IS\textsubscript{CR1}</td>
<td>GATACTAACTGGCGTGACAAGAG</td>
<td>0.97</td>
</tr>
<tr>
<td>P2</td>
<td>\textit{bla\textsubscript{PER-1}}</td>
<td>CTCGTCTCCCTGATACGCTTTC</td>
<td>6.3</td>
</tr>
<tr>
<td>P3</td>
<td>\textit{IntI}</td>
<td>CGAACCCAGTGAGCATAAAGCC</td>
<td>6.3</td>
</tr>
<tr>
<td>P4</td>
<td>\textit{tnpR} of \textit{Tn1696}</td>
<td>GCCCTTCTTTGACGAACTCCA</td>
<td>7.8</td>
</tr>
<tr>
<td>P5</td>
<td>\textit{bla\textsubscript{PER-1}}</td>
<td>CTCGTCTCCCTGATACGCTTTC</td>
<td>4.6</td>
</tr>
<tr>
<td>P6</td>
<td>Downstream of \textit{abct}</td>
<td>GGTAGCGAGATTCCAGACAGA</td>
<td>4.0</td>
</tr>
<tr>
<td>P7</td>
<td>\textit{abct}</td>
<td>CCACACATACACCATACATCC</td>
<td>5.7</td>
</tr>
<tr>
<td>P8</td>
<td>\textit{orf5}</td>
<td>CATCAGCCGCAACGCTGTC</td>
<td>6.4</td>
</tr>
<tr>
<td>P9</td>
<td>\textit{IS6100}</td>
<td>AGGGGCGTCTGCGAAATGGTG</td>
<td>4.0</td>
</tr>
<tr>
<td>P2</td>
<td>\textit{bla\textsubscript{PER-1}}</td>
<td>CTCGTCTCCCTGATACGCTTTC</td>
<td>4.6</td>
</tr>
<tr>
<td>P7</td>
<td>\textit{abct}</td>
<td>CCACACATACACCATACATCC</td>
<td>5.7</td>
</tr>
<tr>
<td>P2</td>
<td>\textit{bla\textsubscript{PER-1}}</td>
<td>CTCGTCTCCCTGATACGCTTTC</td>
<td>6.4</td>
</tr>
<tr>
<td>P7</td>
<td>\textit{abct}</td>
<td>CCACACATACACCATACATCC</td>
<td>6.4</td>
</tr>
</tbody>
</table>

**FIG 1** Genetic environment of IS\textsubscript{CR1}-mediated \textit{bla\textsubscript{PER-1}} in \textit{Acinetobacter johnsonii} XBB1 (GenBank accession no. KF017283) (A), \textit{Aeromonas punctate} 159 (GenBank accession no. GQ891757) (B), and non-O1, non-O139 \textit{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 ISCR1-bla<sub>PER-1</sub>-truncated 3’ CS may function as a powerful genetic vehicle that can facilitate horizontal dissemination of bla<sub>PER-1</sub>.

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<sub>PER-1</sub> is carried on a conjugative and broad-host-range IncA/C plasmid, a Tn1696-like transposon is located in this plasmid, and a highly mobile ISCR1 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<sub>PER-1</sub> in non-O1, non-O139 V. cholerae RJ354 has been deposited in GenBank under the accession number KP076293.

**ACKNOWLEDGMENTS**

This work was supported by the Shanghai Science and Technology Specific Project (grant no. 11ZR142200) and the National Natural Science Foundation of China (grant no. 81472010).

**REFERENCES**