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# *qnrVC*-Like Gene Located in a Novel Complex Class 1 Integron Harboring the IS*CR1* Element in an *Aeromonas punctata* Strain from an Aquatic Environment in Shandong Province, China[▽]

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**A *qnrVC*-like gene, *qnrVC4*, was found in a novel complex class 1 integron gene cassette array following the IS*CR1* element and $bla_{PER-1}$ in a multidrug-resistant strain of the aquatic bacterium *Aeromonas punctata*. The deduced QnrVC4 protein sequence shares 45% to 81% amino acid identity with quinolone resistance determinants QnrB6, QnrA1, QnrS1, QnrC, QnrVC1, and QnrVC3. A Ser-83 to Ile amino acid substitution in gyrase A may be mainly responsible for ciprofloxacin resistance in this strain.**

*Aeromonas* spp. have various class 1 integrons and have been proposed as clinical and environmental reservoirs of antibiotic determinants (2, 9). Resistance to quinolones depends on mutations in the gyrase and/or topoisomerase IV genes (6, 15), and plasmid-mediated resistance determinants (*qnrS2*) have been found in *Aeromonas* spp. (2, 12, 18). Since *qnr* was first detected (11), 6 *qnrA* variants, 20 *qnrB* variants, and 4 *qnrS* variants have been described (7). Two homologs, *qnrVC1* and *qnrVC2*, have been found in integrons (5). Recently, *qnrC* and *qnrD* were isolated in China (3, 20). IS*CR1* elements located downstream of class 1 integrons and transposed by a rolling-circle (RC) transposition mechanism are widespread (14, 17).

In 2008, we investigated the molecular diversity of class 1 integrons among bacteria from wastewater samples near Thousand-Buddha Hill Hospital, Jinan, Shandong province, China. *Aeromonas punctata* 159, identified by its 16S rRNA gene, was selected from kanamycin-containing MacConkey agar plates and characterized as resistant to ampicillin, kanamycin, chloramphenicol, trimethoprim, sulfisoxazole, ceftazidime, and nalidixic acid on Mueller-Hinton agar plates by the disk diffusion method (4). According to the Etest strip manufacturer's data (AB Biodisk, Solna, Sweden), the MICs of ciprofloxacin, gatifloxacin, and nalidixic acid are 0.38, 0.19, and 96 μg/ml, respectively.

Integrase (23) and IS*CR1* (14) were investigated by PCR amplification of genomic DNA extracted using bacterial genome extraction kits (Bioteke, Beijing, China). Amplicons of 3.2 kb and 2.0 kb were obtained using the primers hep58 and hep59 (22) and sequenced. The 2.0-kb amplicon with *dfrA12-orfF-aadA2* is widely distributed (17). The 3.2-kb band contained two *aacA4* cassettes bounding a 218-amino-acid-encoding open reading frame (ORF) following a *catB3* cassette (Fig. 1A). The deduced protein sequence of the ORF included a pentapeptide repeat motif. The new gene showed 99% identity with the nonfunctional *qnrVC2*, which has three nucleotide insertions and one deletion compared to functional *qnr* genes (5, 20), and showed 75%, 75%, 68%, 62%, 60%, 48%, and 34% similarities to *qnrVC1*, *qnrVC3*, *qnrC*, *qnrS1*, *qnrA1*, *qnrB6*, and *qnrD*, respectively, with generally commensurate degrees of protein similarity. The *attC* site of the *qnr*-like gene was analyzed with *qnrVC1* and *qnrVC2* (Fig. 1C), which have been reported to form a superintegron (SI) cassette in *Vibrio parahaemolyticus* and *Vibrio cholerae* (5). The gene was named *qnrVC4*, and its protein was designated QnrVC4 based on the *qnr* nomenclature (7).

Although the *qnrVC4* gene of *A. punctata* was not transferable by transformation of plasmids or conjugation (19), the location of the gene was studied by DNA-DNA hybridizations with probes for the *qnrVC4* gene and for the 23S rRNA gene. Both hybridizations were carried out using plasmid isolation by alkaline lysis (16), followed by agarose gel electrophoresis and total DNA digestion with S1 nuclease and I-CeuI, followed by pulsed-field gel electrophoresis (PFGE) (1, 10). The results indicated that *qnrVC4* was located on a large plasmid (Fig. 2).

Since IS*CR1* was found, primers hep58 and orf341B were used to determine the adjacent region. A 5.3-kb amplicon was obtained and sequenced, and this procedure demonstrated that *aacA4-qnrVC4-aacA4-catB3* is followed by *qacE*Δ*1*, *sul1*, and IS*CR1* (Fig. 1A). The primer aadA2F was used with orf341B to investigate the relationship between *dfrA12-orfF-aadA2* and IS*CR1*, but no amplicon was obtained.

Self-formed adaptor PCR (SEFA-PCR) (21) was used to amplify the region downstream of IS*CR1*, initially using 513Sp1 and 513Sp3, and then 513Sp2 (Table 1) was used for nested PCR. A 3.9-kb region was obtained, and sequencing revealed three ORFs. A 927-bp ORF encoding extended-spectrum β-lactamase PER-1 and a putative promoter were observed upstream of $bla_{PER-1}$ (Fig. 1A). The only other reported $bla_{PER-1}$ associated with IS*CR1* is located downstream of *ant*(*3′*)*-Ij–aac*(*6′*)*Ib–nit1–nit2–catB3* in *A. punc-*

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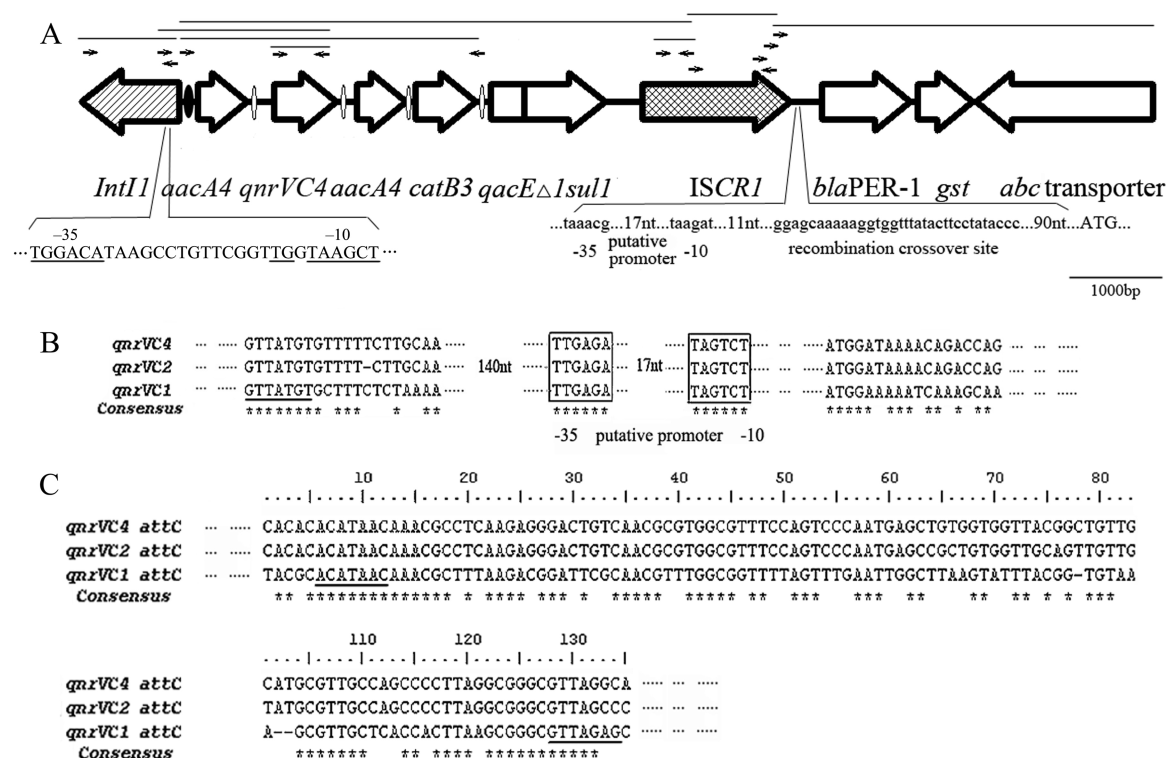


FIG. 1. Schematic map of the complex class 1 integron in *A. punctata* 159. (A) Open arrows indicate open reading frames, white ovals indicate *attC* recombination sites, a black oval indicates the *attI1* recombination site, the arrow with diagonal lines represents the class 1 integrase, and the cross-hatched arrow represents a putative recombinase encoded by IS*CR1* element. The −35 and −10 motifs of the putative promoter in the class 1 integrase are shown in uppercase. The −35 and −10 motifs of the putative promoter of $bla_{PER-1}$, the IS*CR1* recombination crossover sites, and right-hand boundary of the *CR1* element are shown in lowercase. The ATG start codon of the $bla_{PER-1}$ gene is shown in uppercase. The locations of the primers are indicated by small arrows, and PCR products are indicated by lines above the structure. nt, nucleotides; *, conserved residues. (B) Analysis of the putative promoter of the *qnrVC4* gene cassette. (C) *qnrVC4 attC* recombination site analysis showing 91% and 69% identities with *qnrVC2* and *qnrVC1*, respectively.

*tata* integron In39 (AY740681). Most $bla_{PER-1}$ genes are associated with the Tn*1213* transposon backbone and IS*4* family (13). Another 657-bp ORF appears to encode a glutathione *S*-transferase, and the 1.8-kb ORF may encode an ABC transporter.

The *qnrVC4* coding sequence (CDS) was amplified using primers qnrVC4XF and qnrVC4BR, and a 1.8-kb fragment containing the native $P_{ant}$ promoter of *aacA4* and *qnrVC4* was obtained by PCR using the primers Pant-XF and qnrVC4BR, digested with XhoI and BamHI, ligated into pBCKS(+), and transformed into *Escherichia coli* Top10. Recombinants were selected and verified by sequencing. The MICs of ciprofloxacin, gatifloxacin, and nalidixic acid for clones carrying *qnrVC4* were 0.032, 0.047, and 4 μg/ml, respectively, and the corresponding MICs for an alternate insert with a $P_{ant}$ recombinant were 0.008, 0.006, and 2 μg/ml, respectively. As a negative control, *E. coli* Top10 showed MICs of 0.002 μg/ml for ciprofloxacin and gatifloxacin and 0.5 μg/ml for nalidixic acid. $P_{ant}$ of *A. punctata* 159 belonged to $PcW_{TGN-10}$ containing the weak promoter (PcW: −35 TGGACA and −10 TAAGCT) with a TG motif at positions −15 and −14 before the −10 region (Fig. 1A). The $PcW_{TGN-10}$ was 1.7-fold less active than the strong promoter (PcS: −35 TTGACA and −10 TAAACT) and 15-fold more active than PcW (8). Another putative promoter like *qnrVC1* was found in the *qnrVC4* cassette (Fig. 1B). The MIC levels of *qnrVC4* in the integron are probably determined by the promoters and the environment downstream of the *aacA4* cassette.

Therefore, critical regions of *gyrA*, *gyrB*, and *parC*, including quinolone resistance-determining regions (QRDR), were sequenced (with primers listed in Table 1). In the 482-bp *gyrA* amplicon, "X" mutation (random mutation) leads to replacement of a conserved Ser-83 with Ile, which is associated with reduced sensitivity to quinolones in *A. punctata* (6, 15). GyrB QRDR peptides are identical to those of other *Aeromonas* spp. Analysis of ParC QRDR fragments showed that they were the same as those of the sensitive strain *Aeromonas caviae* CIP 7616 (6). The gyrase A subunit containing Ser-83 may form a turn in the secondary structure, but the Leu-83 mutant would form an α-helix (24), which may affect binding of quinolones to the target GyrA.

Quinolone resistance is caused by both topoisomerase mutations and plasmid-mediated determinants in *Aeromonas* spp. The *qnrVC4* cassette shows strong nucleotide similarity to *qnrVC2* (99%), and QnrVC4 shows 81% and 77% identities with QnrVC3 and QnrVC1, respectively, indicating that *qnrVC4* would have originated from *Vibrionaceae*

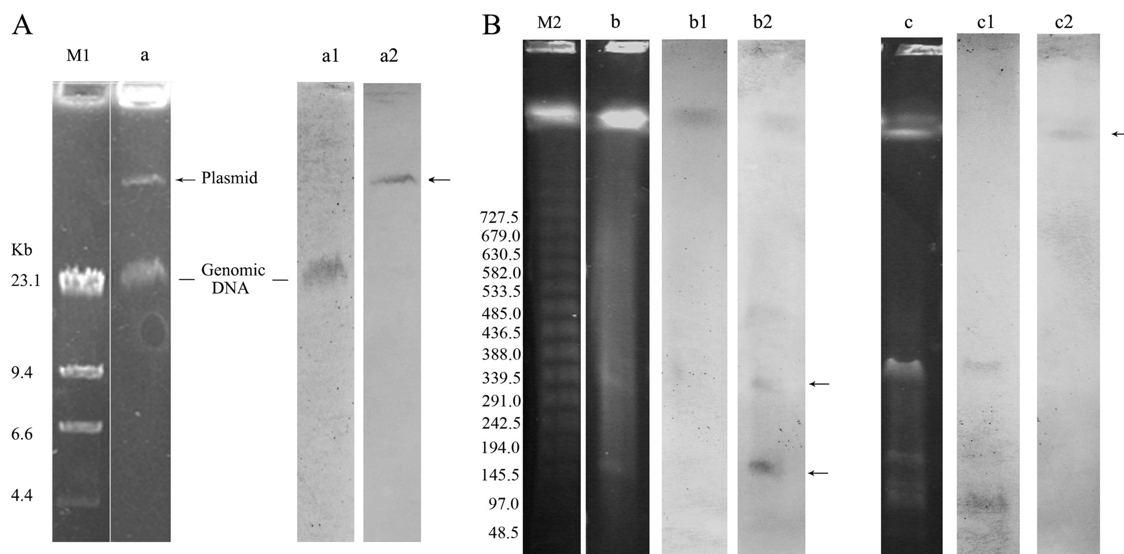


FIG. 2. *qnrVC4* location identified by agarose gel electrophoresis (A), genomic mapping with S1 nuclease and I-CeuI by pulsed-field gel electrophoresis (PFGE) (B), and relative hybridization with the 23S rRNA gene and the *qnrVC4* probe. Lane a shows large-plasmid analysis of *A. punctata* 159; lanes b and c show genomic mapping with S1 nuclease and I-CeuI digestion by PFGE. Lanes a1, b1, and c1 show hybridization results with the 23S rRNA gene. Lanes a2, b2, and c2 show hybridization with the *qnrVC4* probe. M1, HindIII-digested λ DNA marker; M2, λ concatemer marker for PFGE. Arrows indicate plasmid locations.

(5). The *qnrVC4* gene, with its novel gene cassette array and *bla*$_{PER-1}$, is the first *qnrVC* gene associated with *bla*$_{PER-1}$ and a complex class 1 integron in *Aeromonas* spp. These results suggest that there may be further variants of *qnr* genes or new complex structures.

**Nucleotide sequence accession numbers.** The complex class 1 integron sequences and *gyrA*, *gyrB*, and *parC* nucleotide sequences in *A. punctata* 159 have been deposited into the GenBank database with the accession numbers GQ891757, GQ891754, GQ891755, and GQ891756, respectively.

TABLE 1. Primers used in this study

| Primer | Target gene or DNA fragment | Sequence (5′–3′) | PCR product size | Reference |
|---|---|---|---|---|
| IntI1F<br>IntI1R | *intI1* | GTTCGGTCAAGGTTCTGG<br>CGTAGAGACGTCGGAATG | 890 bp | 23 |
| K90<br>K94 | 16S rRNA gene | GAGAGTTTGATCCTGGCTCAG<br>CGGCTACCTTGTTACGACTTC | 1.4 kb | 23 |
| orf341A<br>orf341B | *orf513* | CGCCCACTCAAACAAACG<br>GAGGCTTTGGTGTAACCG | 452 bp | 14 |
| hep58<br>hep59 | Class 1 gene cassette array | TCATGGCTTGTTATGACTGT<br>GTAGGGCTTATTATGCACGC | Variable | 22 |
| aadA2F | | GCTAAGCAAGCTTATCTGGGAC | | This study |
| 513Sp1<br>513Sp3<br>513Sp2 | The region downstream of IS*CR1* | TCGGCCATTCCGACGTCTCTACGA<br>CGCTCACCGCTTGATNNNNNNNNNCCCCTC<br>CATGTGCTGAAAGTTGGCGGTGCC | Variable | This study |
| qnrVC4XF<br>qnrVC4BR | *qnrVC4* | CCCTCGAGCATGGATAAAACAGACCAGTTATA<br>CGGGATCCTTAGTCAGGAACTACTATTAAACCT | 657 bp | This study |
| Pant-XF (with qnrVC4BR) | P$_{ant,}$ *aacA4*, *qnrVC4* | CCCTCGAGCGAAACGGATGAAGGCAC | 1.8 kb | This study |
| gyrAF<br>gyrAR | *gyrA* | TCCTATCTTGATTACGCCATG<br>CATGCCATRCCYACCGCRAW | 482 bp | This study |
| gyrBF<br>gyrBR | *gyrB* | GGGGTCTACTGCTTCACCAA<br>GCATCTGTCATGATGATGATG | 704 bp | This study |
| parCF<br>parCR | *parC* | GTKCAGCGSCGCATCATCTAC<br>CGGTRTAACGCATKGCSGC | 243 bp | This study |

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## REFERENCES


1. **Barton, B. M., G. P. Harding, and A. J. Zuccarelli.** 1995. A general method for detecting and sizing large plasmids. Anal. Biochem. **226:**235–240.
2. **Cattoir, V., L. Poirel, C. Aubert, C. J. Soussy, and P. Nordmann.** 2008. Unexpected occurrence of plasmid-mediated quinolone resistance determinants in environmental *Aeromonas* spp. Emerg. Infect. Dis. **14:**231–237.
3. **Cavaco, L. M., H. Hasman, S. Xia, and F. M. Aarestrup.** 2009. *qnrD*, a novel gene conferring transferable quinolone resistance in *Salmonella enterica* serovar Kentucky and Bovismorbificans strains of human origin. Antimicrob. Agents Chemother. **53:**603–608.
4. **Clinical and Laboratory Standards Institute.** 2007. Performance standards for antimicrobial susceptibility testing; 17th informational supplement M100-S17. Clinical and Laboratory Standards Institute, Wayne, PA.
5. **Fonseca, É. L., F. Dos Santos Freitas, V. V. Vieira, and A. C. P. Vicente.** 2008. New *qnr* gene cassettes associated with superintegron repeats in *Vibrio cholerae* O1. Emerg. Infect. Dis. **14:**1129–1131.
6. **Goñi-Urriza, M., C. Arpin, M. Capdepuy, V. Dubois, P. Caumette, and C. Quentin.** 2002. Type II topoisomerase quinolone resistance-determining regions of *Aeromonas caviae*, *A. hydrophila*, and *A. sobria* complexes and mutations associated with quinolone resistance. Antimicrob. Agents Chemother. **46:**350–359.
7. **Jacoby, G., V. Cattoir, D. Hooper, L. Martínez-Martínez, P. Nordmann, A. Pascual, L. Poirel, and M. Wang.** 2008. *qnr* gene nomenclature. Antimicrob. Agents Chemother. **52:**2297–2299.
8. **Jové, T., S. Da Re, F. Denis, D. Mazel, and M. C. Ploy.** 2010. Inverse correlation between promoter strength and excision activity in class 1 integrons. PLoS Genet. **6:**e1000793.
9. **Lee, M. F., C. F. Peng, Y. H. Lin, S. R. Lin, and Y. H. Chen.** 2008. Molecular diversity of class 1 integrons in human isolates of *Aeromonas* spp. from southern Taiwan. Jpn. J. Infect. Dis. **61:**343–349.
10. **Liu, S. L., A. Hessel, and K. E. Sanderson.** 1993. Genomic mapping with I-*Ceu* I, an intron-encoded endonuclease specific for genes for ribosomal RNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria. Proc. Natl. Acad. Sci. U. S. A. **90:**6874–6878.
11. **Martínez-Martínez, L., A. Pascual, and G. A. Jacoby.** 1998. Quinolone resistance from a transferable plasmid. Lancet **351:**797–799.
12. **Picão, R. C., L. Poirel, A. Demarta, C. S. Silva, A. R. Corvaglia, O. Petrini, and P. Nordmann.** 2008. Plasmid-mediated quinolone resistance in *Aeromonas allosaccharophila* recovered from a Swiss lake. J. Antimicrob. Chemother. **62:**948–950.
13. **Poirel, L., L. Cabanne, H. Vahaboglu, and P. Nordmann.** 2005. Genetic environment and expression of the extended-spectrum beta-lactamase *bla*$_{\mathrm{PER\text{-}1}}$ gene in gram-negative bacteria. Antimicrob. Agents Chemother. **49:**1708–1713.
14. **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*$_{\mathrm{(CTX\text{-}M\text{-}9)}}$. Antimicrob. Agents Chemother. **46:**2656–2661.
15. **Sinha, S., S. Chattopadhyay, S. K. Bhattacharya, G. B. Nair, and T. Ramamurthy.** 2004. An unusually high level of quinolone resistance associated with type II topoisomerase mutations in quinolone resistance-determining regions of *Aeromonas caviae* isolated from diarrhoeal patients. Res. Microbiol. **155:**827–829.
16. **Takahashi, S., and Y. Nagano.** 1984. Rapid procedure for isolation of plasmid DNA and application to epidemiological analysis. J. Clin. Microbiol. **20:**608–613.
17. **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.
18. **Verner-Jeffreys, D. W., T. J. Welch, T. Schwarz, M. J. Pond, M. J. Woodward, S. J. Haig, G. S. Rimmer, E. Roberts, V. Morrison, and C. Baker-Austin.** 2009. High prevalence of multidrug-tolerant bacteria and associated antimicrobial resistance genes isolated from ornamental fish and their carriage water. PLoS One **4:**e8388.
19. **Wang, M., J. H. Tran, G. A. Jacoby, Y. Zhang, F. Wang, and D. C. Hooper.** 2003. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. Antimicrob. Agents Chemother. **47:**2242–2248.
20. **Wang, M., Q. Guo, X. Xu, X. Wang, X. Ye, S. Wu, D. C. Hooper, and M. Wang.** 2009. New plasmid-mediated quinolone resistance gene, *qnrC*, found in a clinical isolate of *Proteus mirabilis*. Antimicrob. Agents Chemother. **53:**1892–1897.
21. **Wang, S., J. He, Z. Cui, and S. Li.** 2007. Self-formed adaptor PCR: a simple and efficient method for chromosome walking. Appl. Environ. Microbiol. **73:**5048–5051.
22. **White, P. A., C. J. McIver, Y. Deng, and W. D. Rawlinson.** 2000. Characterisation of two new gene cassettes, *aadA5* and *dfrA17*. FEMS Microbiol. Lett. **182:**265–269.
23. **Xu, H., J. Davies, and V. Miao.** 2007. Molecular characterization of class 3 integrons from *Delftia* spp. J. Bacteriol. **189:**6276–6283.
24. **Yoshida, H., M. Bogaki, M. Nakamura, and S. Nakamura.** 1990. Quinolone resistance-determining region in the DNA gyrase *gyrA* gene of *Escherichia coli*. Antimicrob. Agents Chemother. **34:**1271–1272.