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MECHANISMS OF RESISTANCE

Molecular Characterization of *qnrVC* Genes and Their Novel Alleles in *Vibrio* spp. Isolated from Food Products in China

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ABSTRACT This study reports the prevalences of *qnrVC* genes in 74 ciprofloxacin-resistant *Vibrio* sp. isolates. Two novel functional *qnrVC* alleles, *qnrVC8* and *qnrVC9*, sharing 98% and 99% nucleotide similarity with *qnrVC6* and *qnrVC7*, respectively, were identified. Our findings suggested that carriage of *qnrVC* alleles, together with target mutations in *gyrA* and *parC* genes, may contribute to the development of fluoroquinolone resistance in *Vibrio* species, posing a serious threat to public health.

KEYWORDS *qnrVC*, *Vibrio*, ciprofloxacin resistance

Vibrio spp. are some of the most important pathogens that cause foodborne illnesses worldwide. Ciprofloxacin is the main choice for the treatment of infections caused by *Vibrio* species. Previous studies have shown that quinolone resistance arises as a result of mutational changes in genes encoding the target bacterial enzymes of fluoroquinolones, namely, DNA gyrase and DNA topoisomerase IV (1–3), and by changes in the levels of expression of efflux pumps and porins that control the accumulation of these agents inside the bacterial cell (4). In 1998, plasmid-mediated quinolone resistance (PMQR) was reported in a clinical isolate of *Klebsiella pneumoniae*, from which low-level quinolone resistance could be transferred to other Gram-negative bacteria (5). The PMQR gene in such an isolate, subsequently named *qnr*, was found to encode a protein that could bind and protect DNA gyrase and topoisomerase IV from inhibition by ciprofloxacin (6). Qnr proteins belong to the pentapeptide repeat family. Until now, six families of Qnr proteins have been described, including QnrA, QnrB, QnrC, QnrD, QnrE, QnrS, and QnrVC (2, 7–11). The *qnrVC* genes, first described in *V. cholerae* in 2008, encodes a pentapeptide repeat protein (PRP) which consists of 218 amino acids (8). *Vibrio* spp. were considered a possible source of *qnr*-like quinolone resistance determinants (12). To date, seven *qnrVC* alleles (*qnrVC1* to *qnrVC7*) have been reported in *Vibrionaceae* (8, 13–15), but the prevalences of *qnrVC* alleles in foodborne *Vibrio* spp. are not well documented.

In this study, a total of 589 nonduplicated isolates of the *Vibrio* spp. were recovered from 801 food samples purchased from open markets and supermarkets in Shenzhen, China, during 2015 and 2016 (Supplemental Materials and Methods). The rates of positivity of various food samples were 89% (shrimp), 24% (pork), 23% (chicken), and 12% (beef), with 379, 149, 51, and 10 *Vibrio* strains isolated from each sample type, respectively. *V. parahaemolyticus* was the most dominant species, accounting for 66% ($n = 386$) of the *Vibrio* sp. isolates tested, followed by *V. alginolyticus* (30% [$n = 175$]), *V. cholerae* (3% [$n = 20$]), and *V. vulnificus* (1% [$n = 8$]) (see Table S1 in the supplemental material).

Antimicrobial susceptibilities were determined for these *Vibrio* isolates as described

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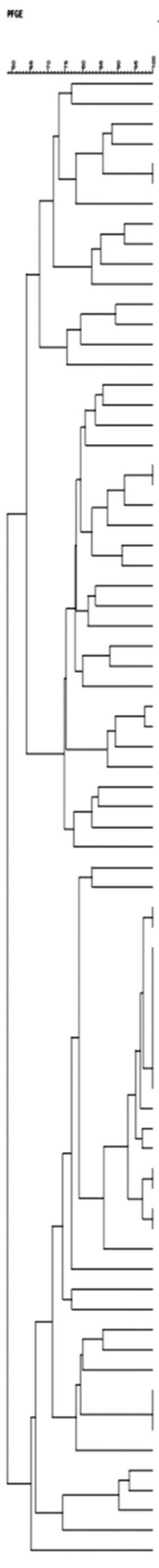
in the Supplemental Materials and Methods. Resistance to ampicillin was the most common among the *Vibrio* spp., with rates of 96%, 77%, 35%, and 38% in *V. parahaemolyticus*, *V. alginolyticus*, *V. cholerae*, and *V. vulnificus*, respectively (Table S2). The rates of resistance to ciprofloxacin among the four species were 9.3%, 17.1%, 40.0%, and 0%, respectively. However, the resistance rates for ofloxacin, at 5.4%, 9.7%, 5.0%, and 0.0%, respectively, for the four species, did not align well with those for ciprofloxacin, suggesting that the mechanisms of resistance to different fluoroquinolones were not identical. Around 20% of the *V. parahaemolyticus* and *V. alginolyticus* isolates exhibited resistance to cephalosporins (ceftriaxone and cefotaxime), whereas the resistance rates were lower (5% and 10%, respectively) in *V. cholerae* but higher in *V. vulnificus* (37.5% and 12.5%, respectively). Resistance to other antibiotics was not high compared to that described in other reports (Table S2). All 74 ciprofloxacin-resistant *Vibrio* sp. isolates were selected for further analysis, among which 37, 30, and 7 isolates were *V. parahaemolyticus*, *V. alginolyticus*, and *V. cholerae*, respectively.

Pulsed-field gel electrophoresis (PFGE) was conducted to study the genetic relationships of the ciprofloxacin-resistant *Vibrio* sp. isolates (Supplemental Materials and Methods). Although signs of clonal dissemination were detectable among organisms collected from different food types on different dates, remarkable genetic diversity can be seen (Fig. 1). Strain Vb624, a *V. parahaemolyticus* strain isolated from a pork sample, was found to exhibit a PFGE pattern identical to those of seven other *V. parahaemolyticus* strains isolated from shrimp samples purchased on different dates. Most of the *Vibrio* strains that exhibited identical PFGE profiles were isolated from either the same sample or different samples on the same date. However, in view of the differences in the antimicrobial susceptibility profiles of these strains, we still consider them different strains.

The 74 ciprofloxacin-resistant *Vibrio* sp. isolates were screened for the presence of *qnrVC* genes (Supplemental Materials and Methods). Thirty-nine of the ciprofloxacin-resistant *Vibrio* sp. isolates tested (53%) were found to harbor the *qnrVC* genes. The rates of positivity among *V. alginolyticus*, *V. parahaemolyticus*, and *V. cholerae* were 63% ($n = 19$), 43% ($n = 16$), and 57% ($n = 4$), respectively. A total of five different *qnrVC* alleles were detected in these isolates, with *qnrVC5* ($n = 18$ [46%]) being the most dominant type, followed by *qnrVC4* ($n = 10$ [26%]), *qnrVC6* ($n = 5$ [13%]), *qnrVC1* ($n = 2$ [5%]), and *qnrVC7* ($n = 1$ [3%]). Additionally, three isolates (Vb132, Vb183, and Vb259) carrying novel variants of *qnrVC* were detected. Strain Vb132, a *V. parahaemolyticus* isolate obtained from shrimp sample, carried an allele that shared 98% nucleotide identity with *qnrVC6*. This allele encodes a protein with the $D^{166}A$ and $K^{185}N$ amino acid substitutions compared with *qnrVC6* and was designated *qnrVC8* (accession no. MH181806). Strain Vb183, a *V. alginolyticus* isolate obtained from a shrimp sample, and strain Vb259, a *V. cholerae* isolate obtained from a pork sample, carried a *qnrVC* variant designated *qnrVC9* (accession no. MH181807). Such a variant exhibited 99% nucleotide similarity to *qnrVC7*, with an $A^{100}V$ substitution (Fig. S1). Both *qnrVC8* and *qnrVC9* were cloned into the pET-15b vector, as described in Supplemental Materials and Methods. Transformants of *Escherichia coli* BL21(DE3) carrying pET15b-*qnrVC8* or pET15b-*qnrVC9* exhibited a 16-fold elevated MIC of ciprofloxacin compared with that for strain *E. coli* BL21(DE3), suggesting that these two *qnrVC* variants may contribute to quinolone resistance (Table 1).

S1-PFGE and hybridization were performed to determine the genetic locations of the *qnrVC* alleles (Supplemental Materials and Methods) and showed that all *qnrVC1* ($n = 2$) genes were located in the chromosome, while all *qnrVC*6 genes ($n = 5$) and the *qnrVC7* gene ($n = 1$) were plasmid borne. The *qnrVC4* and *qnrVC5* genes were detected in both chromosomes and plasmids; among them, 1 out of the 10 *qnrVC4* genes was located in the chromosome, whereas 13 out the 18 *qnrVC5* genes were located in plasmids. The novel *qnrVC* alleles, *qnrVC8* ($n = 1$) and *qnrVC9* ($n = 2$), were all found to reside in the chromosome. Plasmids carrying different *qnrVC* genes varied from ~80 kb to 330 kb in size (Fig. 1). Conjugation experiments were performed on *Vibrio* sp. isolates which carried both chromosome- and plasmid-borne *qnrVC* genes (Supplemental Materials and Methods). Three strains, Vb266 (a *V. parahaemolyticus* strain isolated from



ID	Isolation date	Origin	Species	MIC of CIP	*qnr* genes	Location (kb)	GyrA	ParC
Vb199	2015.10.08	Shrimp1	VA	16	*qnrVC5*	Plasmid (125)	ND	ND
Vb502	2015.11.30	Shrimp5	VA	8	\	\	S83I	S85L
Vb618	2015.12.14	Shrimp3	VA	>16	*qnrVC1*	chromosome	S83I	S85L
Vb637	2016.01.04	Shrimp3	VA	>16	\	\	S83I	S85L
Vb639	2016.01.04	Pork2	VA	>16	\	\	S83I	S85L
Vb640	2016.01.04	Pork3	VA	>16	\	\	S83I	S85L
Vb109	2015.09.14	Pork1	VP	8	*qnrVC5*	Plasmid (150)	ND	ND
Vb392	2015.11.09	Shrimp3	VA	8	\	\	S83I	S85L
Vb507	2015.11.30	Shrimp7	VA	>16	*qnrVC5*	Plasmid (100)	S83I	S85L
Vb620	2015.12.14	Shrimp4	VA	>16	*qnrVC5*	Plasmid (100)	S83I	S85L
Vb673	2016.01.18	Shrimp3	VA	8	\	\	S83I	S85L
Vb503	2015.11.30	Shrimp6	VA	>16	*qnrVC5*	Plasmid (130)	ND	ND
Vb579	2015.12.07	Shrimp2	VA	>16	*qnrVC5*	Plasmid (125)	ND	ND
Vb080	2015.08.31	Shrimp1	VP	8	\	\	S83I	S85L
Vb612	2015.12.14	Shrimp2	VA	4	*qnrVC6*	Plasmid (330)	ND	ND
Vb165	2015.09.21	Chicken1	VA	4	*qnrVC4*	chromosome	S83I	S85L
Vb253	2015.10.19	Shrimp1	VA	8	\	\	S83I	S85L
Vb317	2015.10.26	Shrimp5	VC	4	*qnrVC4*	chromosome	S83I	S85L
Vb183	2015.09.21	Pork1	VC	4	*qnrVC9*	chromosome	S83I	S85L
Vb531	2015.11.30	Shrimp2	VC	4	\	\	S83I	S85L
Vb532	2015.11.30	Shrimp2	VC	4	\	\	S83I	S85L
Vb318	2015.10.26	Shrimp6	VC	4	*qnrVC4*	Plasmid (150)	S83I	S85L
Vb259	2015.10.19	Shrimp2	VA	4	*qnrVC9*	\	S83I	S85L
Vb121	2015.09.14	Pork2	VP	4	*qnrVC4*	chromosome	S83I	S85L
Vb309	2015.10.26	Shrimp4	VA	8	*qnrVC4*	chromosome	S83I	S85L
Vb522	2015.11.30	Chicken1	VA	16	*qnrVC5*	Plasmid (80)	S83I	S85L
Vb530	2015.11.30	Shrimp5	VC	4	*qnrVC4*	chromosome	ND	ND
Vb393	2015.11.09	Pork1	VA	8	*qnrVC4*	chromosome	S83I	S85L
Vb196	2015.10.08	Beef1	VA	4	*qnrVC4*	chromosome	S83I	S85L
Vb225	2015.10.12	Pork1	VA	4	*qnrVC4*	chromosome	ND	ND
Vb526	2015.11.30	Shrimp8	VA	4	*qnrVC4*	chromosome	S83I	S85L
Vb252	2015.10.19	Chicken1	VA	4	*qnrVC5*	chromosome	S83I	S85L
Vb509	2015.11.30	Shrimp1	VA	4	*qnrVC5*	chromosome	S83I	S85L
Vb167	2015.09.21	Chicken2	VA	4	*qnrVC5*	chromosome	S83I	S85L
Vb513	2015.11.30	Pork1	VA	4	*qnrVC5*	chromosome	S83I	S85L
Vb019	2015.08.17	Pork1	VP	4	*qnrVC6*	Plasmid (110)	S83I	S85L
Vb141	2015.09.14	Pork3	VC	8	\	\	S83I	S85L
Vb018	2015.08.17	Pork1	VP	8	*qnrVC6*	Plasmid (110)	S83I	S85L
Vb024	2015.08.17	Shrimp1	VA	8	\	\	S83I	S85L
Vb127	2015.09.14	Shrimp1	VP	8	*qnrVC1*	chromosome	ND	ND
Vb486	2015.11.30	Shrimp2	VP	4	\	\	S83I	S85L
Vb477	2015.11.30	Shrimp1	VP	16	*qnrVC6*	Plasmid (185)	ND	ND
Vb478	2015.11.30	Shrimp1	VP	16	*qnrVC6*	Plasmid (185)	ND	ND
Vb212	2015.10.12	Shrimp1	VP	8	\	\	ND	ND
Vb390	2015.11.09	Shrimp2	VA	8	\	\	ND	ND
Vb500	2015.11.30	Shrimp4	VP	8	\	\	ND	ND
Vb573	2015.12.07	Shrimp1	VA	8	\	\	ND	ND
Vb624	2016.01.04	Pork1	VP	16	\	\	ND	ND
Vb631	2016.01.04	Shrimp1	VP	8	\	\	ND	ND
Vb632	2016.01.04	Shrimp2	VP	8	\	\	ND	ND
Vb664	2016.01.18	Shrimp4	VP	>16	\	\	ND	ND
Vb668	2016.01.18	Shrimp5	VP	>16	\	\	S83I	S85L
Vb288	2015.10.26	Shrimp3	VP	8	\	\	ND	ND
Vb662	2016.01.18	Shrimp3	VP	>16	\	\	ND	ND
Vb490	2015.11.30	Shrimp3	VP	16	\	\	ND	ND
Vb491	2015.11.30	Shrimp3	VP	16	\	\	ND	ND
Vb289	2015.10.26	Shrimp4	VP	8	\	\	ND	ND
Vb290	2015.10.26	Shrimp4	VP	8	\	\	ND	ND
Vb277	2015.10.26	Shrimp2	VP	4	\	\	S83I	S85L
Vb591	2015.12.14	Shrimp1	VP	4	\	\	ND	ND
Vb574	2015.12.07	Shrimp1	VA	>16	\	\	ND	ND
Vb651	2016.01.18	Shrimp1	VP	8	*qnrVC7*	Plasmid (130)	ND	ND
Vb266	2015.10.26	Shrimp1	VP	8	*qnrVC5*	Plasmid (100)	ND	ND
Vb603	2015.12.14	Pork1	VP	8	\	\	S83I	S85L
Vb132	2015.09.14	Shrimp2	VP	8	*qnrVC8*	chromosome	ND	ND
Vb656	2016.01.18	Shrimp2	VP	8	*qnrVC5*	Plasmid (125)	ND	ND
Vb660	2016.01.18	Shrimp3	VP	8	*qnrVC5*	Plasmid (125)	ND	ND
Vb667	2016.01.18	Shrimp5	VP	8	*qnrVC5*	Plasmid (125)	S83I	S85L
Vb483	2015.11.30	Pork1	VP	4	\	\	S83I	S85L
Vb062	2015.08.24	Shrimp1	VP	16	*qnrVC5*	Plasmid (125)	ND	ND
Vb293	2015.10.26	Shrimp1	VP	>16	*qnrVC5*	Plasmid (110)	ND	ND
Vb652	2016.01.18	Shrimp1	VP	>16	*qnrVC5*	Plasmid (170)	ND	ND
Vb492	2015.11.30	Shrimp3	VP	4	\	\	S83I	S85L
Vb382	2015.11.09	Shrimp1	VP	8	\	\	S83I	S85L

FIG 1 Genetic and phenotypic characteristics of 74 ciprofloxacin (CIP)-resistant foodborne isolates of *Vibrio* species. The format of the isolation date is year.month.date. VP, *V. parahaemolyticus*; VA, *V. alginolyticus*; VC, *V. cholerae*; VV, *V. vulnificus*; ND, not done; ID, identification.

TABLE 1 Susceptibilities of *Vibrio* sp. isolates and the corresponding *E. coli* transformants and transconjugants to fluoroquinolones

Bacterial strain (variant carried)[a]	Bacterial species	MIC (μg/ml)	
		Ciprofloxacin	Nalidixic acid
BL21(DE3)	*E. coli*	0.0075	1
BL21(DE3)/pET15b	*E. coli*	0.0075	1
BL21(DE3)/pET15b-*qnrVC8*	*E. coli*	0.12	16
BL21(DE3)/pET15b-*qnrVC9*	*E. coli*	0.12	16
Vb132 (*qnrVC8*)	*V. parahaemolyticus*	8	>64
Vb183 (*qnrVC9*)	*V. cholerae*	4	>64
Vb259 (*qnrVC9*)	*V. parahaemolyticus*	4	>64
J53 AZ^R	*E. coli*	0.015	2
Vb266	*V. parahaemolyticus*	8	32
TC-Vb266	*E. coli*	0.5	16
Vb507	*V. alginolyticus*	>16	>64
TC-Vb507	*E. coli*	0.5	16
Vb620	*V. alginolyticus*	>16	>64
TC-Vb620	*E. coli*	0.5	16

[a]TC, transconjugant.

shrimp sample and that carried *qnrVC5*), Vb507 (a *V. alginolyticus* strain isolated from shrimp sample and that carried *qnrVC5*), and Vb620 (a *V. alginolyticus* strain isolated from shrimp sample and that carried *qnrVC5*), were able to transfer the quinolone resistance phenotype to *E. coli* strain J53 AZ^R. All transconjugants exhibited a 32-fold reduction in susceptibility to ciprofloxacin compared with *E. coli* J53 AZ^R (Table 1).

Forty randomly selected ciprofloxacin-resistant *Vibrio* sp. isolates which harbored the *qnrVC* gene were subjected to screening of mutations in the quinolone resistance-determining region (QRDR) of known resistance genes (Supplemental Materials and Methods). As shown in Fig. 1, no mutations were found in the *gyrB* and *parE* genes of the 40 isolates tested. A mutation at codon 83 of the *gyrA* gene that resulted in a Ser-to-Ile substitution and the Ser-to-Leu change at residue 85 of the *parC* gene were identified in all 40 test isolates.

In summary, we screened the *qnrVC* gene and determined the mutations in the quinolone resistance-determining regions of target genes in ciprofloxacin-resistant foodborne isolates of *Vibrio* species. Two novel *qnrVC* alleles (*qnrVC8* and *qnrVC9*) were identified. The findings in this work expand our knowledge on the molecular basis of quinolone resistance among foodborne *Vibrio* species.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00529-18.

SUPPLEMENTAL FILE 1, PDF file, 1.1 MB.

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