Supplementary materials

Molecular characterization of *qnrVC* and their novel alleles in *Vibrio* spp. isolated from food products in China

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Running title: *qnrVC* and novel alleles in foodborne *Vibrio* spp

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Supplementary Materials and methods

Isolation and identification of *Vibrio spp.*

Different food samples, including fresh shrimp, pork, chicken and beef, were purchased from farmers' markets and supermarkets in Shenzhen, Guangdong Province, China during the period August 2015 to January 2016. *Vibrio spp.* strains were isolated according to methods described previously (1). Up to four suspected colonies from each sample were recovered from thiosulfate citrate-bile salts-sucrose agar and were identified by MALDI-TOF MS using a Bruker MicroFlex LT mass spectrometer (Bruker Daltonics).

Antimicrobial susceptibility testing

All strains of *Vibrio spp.* were subjected to antimicrobial susceptibility test using the standard agar dilution method as described by the Clinical and Laboratory Standards Institute (2). Fourteen antimicrobials were tested: ampicillin, tetracycline, amikacin, sulfamethoxazole, cefoxitin, ceftriaxone, cefotaxime, meropenem, nalidixic acid, ciprofloxacin, ofloxacin, amoxicillin, chloramphenicol and gentamicin. *Escherichia coli* strain ATCC25922 and *Staphylococcus aureus* strain ATCC29213 were used as the quality control strains. Strains of the same *Vibrio* spp. isolated from the same food samples and displayed identical antimicrobial susceptibility profiles were considered as the same clone and eliminated from further characterization. The remaining strains were considered as non-duplicate isolates.

Detection of *qnrVC* genes and mutations in QRDRs

Genomic DNA of each *Vibrio* spp. isolate was prepared using the boiling method as previously described (3). The *qnrVC* genes were amplified by PCR with primer pairs targeting *qnrVC1*, *3*, *6* (*qnrVC136*-F-CAGGTAAATGRTAGTCTTCA, *qnrVC136*-R-TTTGTTATGTGCGTAGCC) and *qnrVC4*, *5*, *7*, (*qnrVC457*-F-

ACTCAAATAGAAAGAGGGCTAG, *qnrVC457*-R- TTGAGGCGTTTGTTATGTG). Mutations in the QRDRs of genes encoding DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) in 40 randomly selected *Vibrio* spp. isolates were determined by PCR amplification as described previously (3, 4). PCR products were sequenced, followed by BLASTN analysis to determine the genetic identity.

PFGE subtyping, conjugation, S1-PFGE and hybridization

Pulsed-field gel electrophoresis (PFGE) was conducted to investigate the genetic relatedness of the identified ciprofloxacin-resistant *Vibrio* spp. isolates with methods described previously. Conjugation assays were performed as previously described to test the transferability of the *qnrVC* genes, utilizing J53 AZ^R as the recipient strain. Transconjugants were recovered from LB plates supplemented with 0.5μg/mL ciprofloxacin and 100μg/mL sodium azide. S1-PFGE and Southern hybridization with the *qnrVC* probes were performed on both the parental strain and their corresponding transconjugants as previously described (1) .

Cloning of *qnrVC8* and *qnrVC9*

To confirm the role of the *qnrVC* variant genes *qnrVC8* and *qnrVC9*, cloning experiments were performed as described previously (5). Briefly, DNA segments were amplified using primer pairs targeting the putative novel quinolone resistance genes *qnrVC8* (*qnrVC8*-*Sac*I-F-GATCGAGCTCCAGGTAAATGRTAGTCTTCA, *qnrVC8*- *BamH*I R-TCAGGGATCCTTTGTTATGTGCGTAGCC) and *qnrVC9* (*qnrVC9*- *Sac*I -F-GATCGAGCTCACTCAAATAGAAAGAGGGCTAG, *qnrVC9*- *BamH*I -R-TCAGGGATCCTTGAGGCGTTTGTTATGTG) and their flanking sequences, respectively. The PCR products were digested with restriction enzymes *BamH*I and *Sac*I, and ligated with

a modified cloning vector pET15b, yielding pET15b-*qnrVC8* and pET15b-*qnrVC9*, which were then used to transform *E. coli* DH5α by electroporation. Transformants were selected on LB plates containing 100 μg/mL ampicillin, followed by confirmation of genetic identity through PCR screening with the pair of cloning primers described above. Finally, the recombinant plasmid extracted from DH5α cell was transformed into competent cells of *E. coli* BL21(DE3) and selected on LB agar (with 100 μg/mL ampicillin) again, and the MIC of CIP of BL21(DE3) carrying novel *qnrVC* genes was determined, with *E. coli* BL21(DE3) cells carrying the vector pET15b being used as control. Antimicrobial susceptibility test for *qnrVC8*- and *qnrVC9*-bearing clinical isolates, and the corresponding *E. coli* transformants, was performed by the broth microdilution method, following the CLSI guidelines (6). IPTG was added into MHA broth to induce the expression of *qnrVC* genes during MIC experiment.

Supplementary Tables and figures

Supplementary Table S1. Information of *Vibrio* spp. isolated from different food samples.

Food sample and *Vibrio* strains isolated	**Types of food samples**				**Total**
	Shrimp	**Pork**	**Chicken**	**Beef**	
Sample surveyed	123	465	139	74	801
Positive Samples	109	112	32	9	262
Positive rate (%)	89	24	23	12	33
No. of strains isolated					
V. parahaemolyticsu	252	95	36	3	386
V. alginolyticus	113	42	14	6	175
V. cholerae	11	7	1	1	20
V. vulnificus	3	5	0	0	8
Total	379	149	51	10	589

Supplementary Table S2. Antimicrobial susceptibilities of different species of *Vibrio* strains of food origin

Antibiotics	Break Points (μg/ml)	Resistance rate (%)				
		All strains (n=589)	VP (n=386)	VA (n=175)	VC (n=20)	VV (n=8)
AMP	32	87.4	95.9	77.1	35.0	37.5
AMO	32	13.9	12.7	16.0	20.0	12.5
CRO	4	18.7	19.7	17.1	5.0	37.5
CTX	4	17.7	19.2	15.4	10.0	12.5
MRP	4	0.0	0.0	0.0	0.0	0.0
NAL	32	15.8	11.1	22.9	45.0	12.5
OFL	8	6.6	5.4	9.7	5.0	0.0
CIP	4	12.6	9.3	17.1	40.0	0.0
TET	16	12.1	13.7	9.7	0.0	12.5
AMK	64	0.0	0.0	0.0	0.0	0.0
CHL	32	3.7	2.6	6.3	5.0	0.0
GEN	16	2.7	3.6	0.6	5.0	0.0
SUL	4/76	29.9	26.4	37.7	35.0	12.5

AMP, Ampicillin; AMO, Amoxicillin / clavulanic acid; CRO, Ceftriaxone; CTX, Cefotaxime; MRP, Meropenem; NAL, Nalidixic acid; OFL, Ofloxacin; CIP, Ciprofloxacin; TET, Tetracycline; AMK, Amikacin; CHL, Chloramphenicol; GEN, Gentamicin; SUL, Sulfamethoxazole. VP, *V. parahaemolyticus*; VA, *V. alginolyticus*; VC, *V. cholerae*; VV, *V. vulnificus*.

Supplementary Figure S1. Sequence alignment of eight *qnrVC* alleles. The residues labeled in red or blue were respectively designated the sites in Qnr8 and Qnr9, which were different from other QnrVC proteins.

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