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Characterization of VCC-1, a Novel Ambler Class A Carbapenemase from *Vibrio cholerae* Isolated from Imported Retail Shrimp Sold in Canada

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One of the core goals of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) is to monitor major meat commodities for antimicrobial resistance. Targeted studies with methodologies based on core surveillance protocols are used to examine other foods, e.g., seafood, for antimicrobial resistance to detect resistances of concern to public health. Here we report the discovery of a novel Ambler class A carbapenemase that was identified in a nontoxigenic strain of *Vibrio cholerae* (N14-02106) isolated from shrimp that was sold for human consumption in Canada. *V. cholerae* N14-02106 was resistant to penicillins, carbapenems, and monobactam antibiotics; however, PCR did not detect common β-lactamases. Bioinformatic analysis of the whole-genome sequence of *V. cholerae* N14-02106 revealed on the large chromosome a novel carbapenemase (referred to here as VCC-1, for *Vibrio cholerae* carbapenemase 1) with sequence similarity to class A enzymes. Two copies of bla_{VCC-1} separated and flanked by IS*Vch9* (i.e., 3 copies of IS*Vch9*) were found in an acquired 8.5-kb region inserted into a VrgG family protein gene. Cloned bla_{VCC-1} conferred a β-lactam resistance profile similar to that in *V. cholerae* N14-02106 when it was transformed into a susceptible laboratory strain of *Escherichia coli*. Purified VCC-1 was found to hydrolyze penicillins, 1st-generation cephalosporins, aztreonam, and carbapenems, whereas 2nd- and 3rd-generation cephalosporins were poor substrates. Using nitrocefin as a reporter substrate, VCC-1 was moderately inhibited by clavulanic acid and tazobactam but not EDTA. In this report, we present the discovery of a novel class A carbapenemase from the food supply.

Carbapenems are potent wide-spectrum antibiotics that are routinely used as last-line drugs to treat resistant bacterial infections. Their continued use is threatened by carbapenemases, which are β-lactamases with an extended spectrum of activity against carbapenems. This family of enzymes is divided into two groups on the basis of their enzymatic mechanism; class A and D enzymes utilize an active-site serine where catalysis proceeds through an acyl-enzyme intermediate, whereas class B enzymes are metalloenzymes that promote the direct hydrolysis of the β-lactam via a metal cofactor (1). Carbapenemases are a major determinant of carbapenem resistance, and public health surveillance efforts have found that their spread has accelerated worldwide over the last decade (2). The incidence of carbapenem-resistant *Enterobacteriaceae* (CRE) is increasing in health care settings, and useful drugs to treat them are dwindling (3, 4). CRE possess resistance elements that are often highly mobile and can transfer between species and asymptomatic carriers (5, 6), making CRE outbreaks both logistically and epidemiologically difficult to control.

Antibiotic resistance surveillance efforts have largely focused on pathogens isolated from human specimens to identify and track emerging resistance determinants. The environment has tremendous microbial diversity and is a major reservoir for antimicrobial resistance (7, 8); however, it is not subjected to the same level of surveillance as human clinical isolates, and the risk of antimicrobial-resistant infections from such reservoirs is unclear (9, 10). Antibiotic-resistant organisms have been reported from diverse food sources, including raw and processed foods and various plant and animal sources (11–14). A survey of bacterial isolates from seafood and meat collected from Canadian retail sources found carbapenem-resistant organisms with known mechanisms of action, particularly in seafood (12). Recently, Bier and colleagues identified a nontoxigenic *Vibrio cholerae* isolate that harbored a carbapenemase that could not be identified by standard PCR typing (15). In this work, we present the discovery and characterization of a novel Ambler class A carbapenemase found in a nontoxigenic *V. cholerae* strain isolated from a shrimp intended for human consumption in Canada.

MATERIALS AND METHODS

Source of isolate. *V. cholerae* N14-02106 was collected as part of a targeted study of the carbapenem resistance of *Enterobacteriaceae* found in imported seafood collected through the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) retail food sampling framework in 2014 and described in detail elsewhere (N. Janecko, S. Martz, B. P. Avery, D. Daignault, A. Desruisseau, D. Boyd, R. J. Irwin, M. R. Mulvey, and R. J. Reid-Smith, submitted for publication). The study used selective media to assess the distribution of carbapenemase-producing *Enterobacteriaceae* in seafood. The bacterium was isolated from frozen farmed black tiger shrimp imported from India and purchased in Ontario,

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Canada. Briefly, the shrimp sample was incubated overnight in buffered peptone water. *V. cholerae* N14-02106 was isolated by directly plating the incubated sample onto chromID Carba medium (bioMérieux, Saint-Laurent, QC, Canada).

Using a disk diffusion test, the isolate had an ertapenem disk (10 μg) diameter of ≤25 mm on a Mueller-Hinton plate, which was the cutoff value for a putative carbapenemase producer in the pilot study. *V. cholerae* N14-02106 was also positive for carbapenemase production using the Carba NP test (16). The isolate was identified as *V. cholerae* by the Vitek 2 Compact system, and its identity was confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker Daltonics, Wissembourg, France). Serotyping was performed using antisera against the O1 and O139 serogroups produced by the National Microbiology Laboratory in a standard slide agglutination assay.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was carried out using a Vitek 2 system and the Etest (bioMérieux Canada Inc., St. Laurent, QC, Canada) and a Sensititre automated microbiology system (Trek Diagnostic Systems Ltd., Thermo Fisher Scientific, Oakwood Village, OH, USA) according to the manufacturers' instructions. The antimicrobial susceptibilities of the isolates were interpreted according to the latest CLSI breakpoints.

Whole-genome sequencing and assembly. *V. cholerae* N14-02106 genomic DNA was purified using a MasterPure Complete RNA and DNA purification kit (Epicentre, Madison, WI, USA) according to the manufacturer's instructions. DNA was sequenced by use of both the MiSeq (Illumina, San Diego, CA, USA) and single-molecule real-time sequencing (RSII; Pacific Biosciences, Menlo Park, CA, USA) platforms. The Illumina DNA libraries were prepared with a TruSeq DNA PCR sample preparation kit, and adapter-ligated libraries were size selected for a 500- to 800-bp insert using a Sage Science Blue Pippin instrument (Beverley, MA, USA). Paired-end reads were produced with a MiSeq reagent kit (v3; 600 cycles). Raw reads were preprocessed with FLASH software (17), and *de novo* assembly was performed by use of the SPAdes algorithm (18). Pacific Biosciences DNA libraries with a 20-kb fragment size were prepared, and sequencing was performed by P6-C4 polymerase/chemistry with one single-molecule real-time (SMRT) cell run for 240 min. A draft assembly was produced using the HGAP (v3.0) protocol (19) via the SMRT portal software (v2.3.0).

Bioinformatic analysis. Contigs from the Illumina assembly were searched, using the Comprehensive Antibiotic Resistance Database (http://arpcard.mcmaster.ca), to identify putative β-lactamase genes (20). Annotation was carried out by manual NCBI BLAST analysis (21).

PCR and cloning of bla_{VCC-1}. A multiplex PCR was used to detect the KPC, NDM, GES, VIM, IMP, SME, and OXA-48 β-lactamase genes (22). Primers VibCACF (5′-TTAGCTTATGTGTCCAACCA) and VibCACR (5′-TCTATACAGTTTGTACGACC) were used to amplify bla_{VCC-1}, and the product was cloned into plasmid pCR-XL-TOPO to produce pVCC-1. For VCC-1 production, the bla_{VCC-1} gene was amplified with primers VCCstart (5′-CCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGAAACGTATTGCTATG) and VCCstop (5′-TCTCAAGCTTTCACTTTACATTTTCTATTGCAAT), and the product was cloned with XbaI and HindIII into pET-28b(+) to produce pET-VCC-1.

DNA transfer by conjugation. *Escherichia coli* J53AZR, resistant to sodium azide, was used as the recipient in mating experiments with *V. cholerae* N14-02106. Mating was done in LB broth at 25°C and 30°C with selection for transconjugants on LB agar containing 0.125 μg/ml meropenem and 150 μg/ml sodium azide.

VCC-1 protein purification. Plasmid pET-VCC-1in *E. coli* BL21(DE3) was grown aerobically at 37°C in Terrific Broth supplemented with 25 μg/ml kanamycin to an A_{600} of ≈0.6, induced with 100 μM IPTG (isopropyl-β-D-thiogalactopyranoside), and outgrown for 22 h at 18°C. Cells were harvested by centrifugation at 3,000 × *g* for 10 min, and the periplasm was extracted by resuspending cell pellets in a 1/40 volume of TSE buffer (500 mM sucrose, 200 mM Tris, pH 8, 1 mM EDTA) as described previously (23). Periplasmic extracts were diluted 5-fold with 50 mM sodium acetate buffer (pH 5), loaded onto a 1-ml HiTrap SP XL cation-exchange column (GE Healthcare, Piscataway, NJ, USA) that had been preequilibrated with 50 mM MES (morpholineethanesulfonic acid; pH 6), and eluted with a linear gradient of 0 to 300 mM NaCl. Fractions containing VCC-1 were identified by SDS-PAGE, pooled, buffer exchanged using a centrifugal filter into 50 mM sodium phosphate (pH 7) containing 10% glycerol, and stored at −80°C. The intrinsic molar absorptivity of the mature VCC-1 polypeptide was used to calculate the concentration of protein preparations according to the method of Pace and Schmid (24). The purity of VCC-1 was determined by SDS-PAGE, and the VCC-1 was judged to be greater than 90% pure. The signal peptide cleavage site was determined using the SignalP (v4.1) server (http://www.cbs.dtu.dk/services/SignalP/) (25).

Enzyme kinetics. Imipenem, meropenem, ertapenem, and aztreonam were purchased from Cedarlane Laboratories (Burlington, ON, Canada), and all other antibiotics were purchased from Sigma-Aldrich (St. Louis, MO, USA). VCC-1 activity was measured by monitoring the change in UV absorbance caused by β-lactam ring opening following hydrolysis using an Agilent Cary 50 spectrophotometer (Santa Clara, CA, USA). Reactions were carried out at 22°C in 50 mM sodium phosphate (pH 7). The concentration VCC-1 varied from 0.5 nM to 20 nM, and substrates were tested at 6 to 8 concentrations typically ranging from 0.5× K_m to 5 to 10× K_m. The means of duplicate reactions were reported, and the ranges over the means for both K_m and V_{max} were less than 10%. K_m and V_{max} were determined by fitting the initial reaction rates by nonlinear regression to a rectangular hyperbola using SigmaPlot (v11.0) software. The following extinction coefficients were used for rate measurements: for benzylpenicillin, $\Delta\varepsilon_{232}$ was −1,230 M^{-1} cm^{-1}; for oxacillin, $\Delta\varepsilon_{260}$ was 280 M^{-1} cm^{-1}; for cephalothin, $\Delta\varepsilon_{262}$ was −6,250 M^{-1} cm^{-1}; for cefoxitin, $\Delta\varepsilon_{262}$ was −6,860 M^{-1} cm^{-1}; for cefotaxime, $\Delta\varepsilon_{265}$ was −6,730 M^{-1} cm^{-1}; for ceftazidime, $\Delta\varepsilon_{260}$ was −10,400 M^{-1} cm^{-1}; for aztreonam, $\Delta\varepsilon_{318}$ was −640 M^{-1} cm^{-1}; for imipenem, $\Delta\varepsilon_{299}$ was −10,550 M^{-1} cm^{-1}; and for meropenem, $\Delta\varepsilon_{298}$ was −11,500 M^{-1} cm^{-1}. Inhibitor potency was measured using nitrocefin as a reporter substrate at 490 nm on a Molecular Devices SpectraMax 384 plate reader (Sunnyvale, CA, USA). VCC-1 (0.5 nM) and inhibitor (0.1 to 1000 μM) were preincubated for 10 min, and reactions were initiated by the addition of substrate. Fifty percent inhibitory concentrations were determined by fitting initial rates by nonlinear regression to a four-parameter logistic equation. K_is were determined using the Cheng-Prusoff equation with correction using a competitive model of inhibition; the means and standard deviations of three reactions are reported (26).

Nucleotide sequence accession number. The region harboring bla_{VCC-1}, as described here, was deposited in GenBank (accession number KT818596).

RESULTS

Phenotypic and molecular testing of *V. cholerae* N14-02106. *V. cholerae* N14-02106 grew on the selective medium chromID Carba and was subsequently identified to be a carbapenemase producer by Carba NP testing. In antimicrobial susceptibility tests, the isolate was resistant to ampicillin, amoxicillin-clavulanic acid, cefazolin, cefoxitin, and meropenem but susceptible to ceftiofur, cefpodoxime, cefotaxime, ceftazidime, ceftriaxone, amikacin, gentamicin, tobramycin, ciprofloxacin, tigecycline, chloramphenicol, nitrofurantoin, sulfisoxazole, and trimethoprim-sulfamethoxazole. A PCR screen for KPC, NDM, OXA-48, VIM, IMP, and GES-type β-lactamase genes was negative. Limited serotyping showed *V. cholerae* N14-02106 to be non-O1 and non-O139.

Identification of bla_{VCC-1} from whole-genome sequence. In order to identify the mechanism of carbapenem resistance, *V. cholerae* N14-02106 was subjected to whole-genome sequencing. An assembly of reads from the Illumina sequencing produced 100 contigs with an average depth of coverage of 164 times and an N_{50}

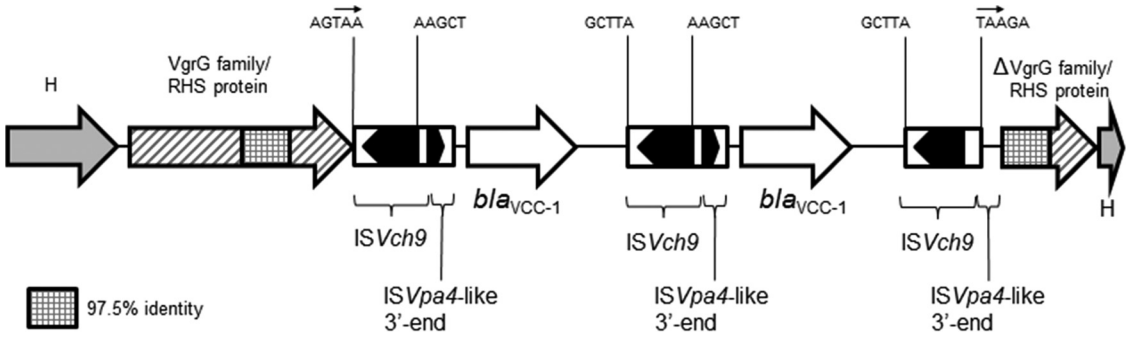

FIG 1 Schematic map of the region containing bla_{VCC-1} on the chromosome of *V. cholerae* N14-02106 (GenBank accession number KT818596). Block arrows, the direction of transcription of the genes; H, hypothetical protein. The sequences of the 5 bp immediately flanking each terminal inverted repeat of the three copies of IS*Vch9* are shown. The putative direct repeats of TAA flanking the acquired region are indicated by small arrows within the 5-bp sequences.

contig length of 126,070 bp. Analysis of these contigs using the Resistance Gene Identifier tool on the CARD website identified a β-lactamase with 58% identity to the IMI-1 carbapenemase located on a 2,727-bp contig. An 855-bp open reading frame (encoding 284 amino acids) was identified and labeled bla_{VCC-1} (where VCC-1 represents *Vibrio cholerae* carbapenemase 1). Sequencing of the N14-02106 genome using the Pacific Biosciences platform produced 952 Mbp of reads with an N_{50} read length of 17,372 bp. *De novo* assembly produced two chromosomes of 2,933,987 bp and 1,228,771 bp with an average depth of coverage of 184 times. No plasmids were detected by sequencing. The bla_{VCC-1} gene was found in an 8.5-kb region on the large chromosome and was inserted into the 3′ end of the virulence-associated *vgrG* gene. Two identical copies of bla_{VCC-1} were found in a head-to-tail arrangement, with each copy being surrounded by a novel insertion sequence designated IS*Vch9* in the ISfinder database (27) (Fig. 1). Beginning 14 bp upstream of the bla_{VCC-1} start codon is a 534-bp remnant of the 3′ end of an IS*Vpa4*-like element (27), which presumably contains sequences that drive the expression of bla_{VCC-1}. The 8.5-kb region described above is flanked by 3-bp direct repeats (Fig. 1) and resembles a novel composite transposon; thus, this region may have been acquired by a transposition mechanism. Attempts to transfer chromosomal bla_{VCC-1} to *E. coli* by conjugation were unsuccessful.

Comparison of the VCC-1 sequence with the sequences of other β-lactamases. A dendrogram using the VCC-1 amino acid sequence aligned with the sequences of select Ambler class A β-lactamases revealed that it is most closely related to the IMI, NMC-A, and SFC-1 carbapenemases, sharing 59.1%, 59.1%, and 58.3% amino acid sequence identity, respectively (Fig. 2). An alignment of the VCC-1 amino acid sequence with the amino acid sequences of KPC-2, NMC-A, SME-1, and IMI-1 is presented in Fig. 3, where the sequence numbering follows the Ambler scheme for class A β-lactamases (28). VCC-1 contains four conserved motifs of class A β-lactamases: ^{70}S(S/T)FK^{73} ^{130}SDN^{132}, ^{166}EXXXN^{170}, and ^{234}KTG^{236}. It also contains in other positions residues that are characteristic of carbapenemases, namely, ^{69}C, ^{238}C, ^{105}H, ^{168}L, ^{237}T, and ^{241}Y (29–31).

VCC-1 is a carbapenemase. Etest results showed that *V. cholerae* N14-02106 was resistant to aztreonam (MIC, >256 μg/ml) and all four carbapenems tested (imipenem, meropenem, ertapenem, and doripenem; MICs, >32 μg/ml) (Table 1). Further, the isolate exhibited intermediate susceptibility to amoxicillin-clavulanic acid, piperacillin-tazobactam, and cefoxitin but was susceptible to 3rd- and 4th-generation cephalosporins. Similar results were obtained by testing with the Vitek 2 and Sensititre systems. Importantly, *E. coli* TOP10 harboring pVCC-1 exhibited resistance to imipenem and ertapenem and intermediate susceptibility to meropenem and doripenem, indicating that VCC-1 is a carbapenemase. Like the clinical isolate, the pVCC-1 transformant was resistant to amoxicillin-clavulanic acid and aztreonam but susceptible to the 3rd- and 4th-generation cephalosporins; however, MICs were generally lower (except for ceftazidime), as is common for laboratory strains of *E. coli* harboring cloned β-lactamase genes (32–36).

Biochemical characterization of VCC-1. Purified VCC-1 exhibited broad and varied hydrolytic activity toward penicillin, monobactam, and carbapenem antibiotics (Table 2). The highest catalytic turnover (k_{cat}) was observed with oxacillin, cephalothin, and imipenem at 153.3 s^{-1}, 320.1 s^{-1}, and 190.2 s^{-1}, respectively. VCC-1 showed reduced activity toward 2nd- and 3rd-generation cephalosporins, and K_ms were too large to be measured. The K_m for meropenem was the lowest at 70.1 μM; however, its turnover rate was a modest 11.2 s^{-1}. VCC-1 showed the highest catalytic efficiency (k_{cat}/K_m) against oxacillin. Inhibitor studies showed that VCC-1 was inhibited by tazobactam and clavulanic acid, with K_is being 2.0 ± 0.4 μM and 13 ± 3 μM, respectively. No evidence of inhibition by EDTA was observed up to a concentration of 1 mM.

DISCUSSION

The novel carbapenemase VCC-1 was identified in a non-O1, non-O139 strain of *V. cholerae* with high-level resistance to carbapenems, and this activity was transferable to a susceptible *E. coli* strain. An alignment of the VCC-1 amino acid sequence with the amino acid sequences of previously characterized carbapenemases revealed signature features of Ambler class A β-lactamases and residues that are indicative of carbapenemase activity. Finally, the VCC-1 protein was able to hydrolyze both meropenem and imipenem *in vitro*, showing kinetic parameters similar to those of the related enzymes IMI-1 and SFC-1, where hydrolysis of meropenem was approximately 10-fold lower than that of imipenem (36). Additionally, the pattern of hydrolytic activity of this enzyme mirrored that of related class A enzymes, where 2nd- and 3rd-generation cephalosporins are generally poorer substrates than 1st-generation cephalosporins, penicillins, monobactams, and

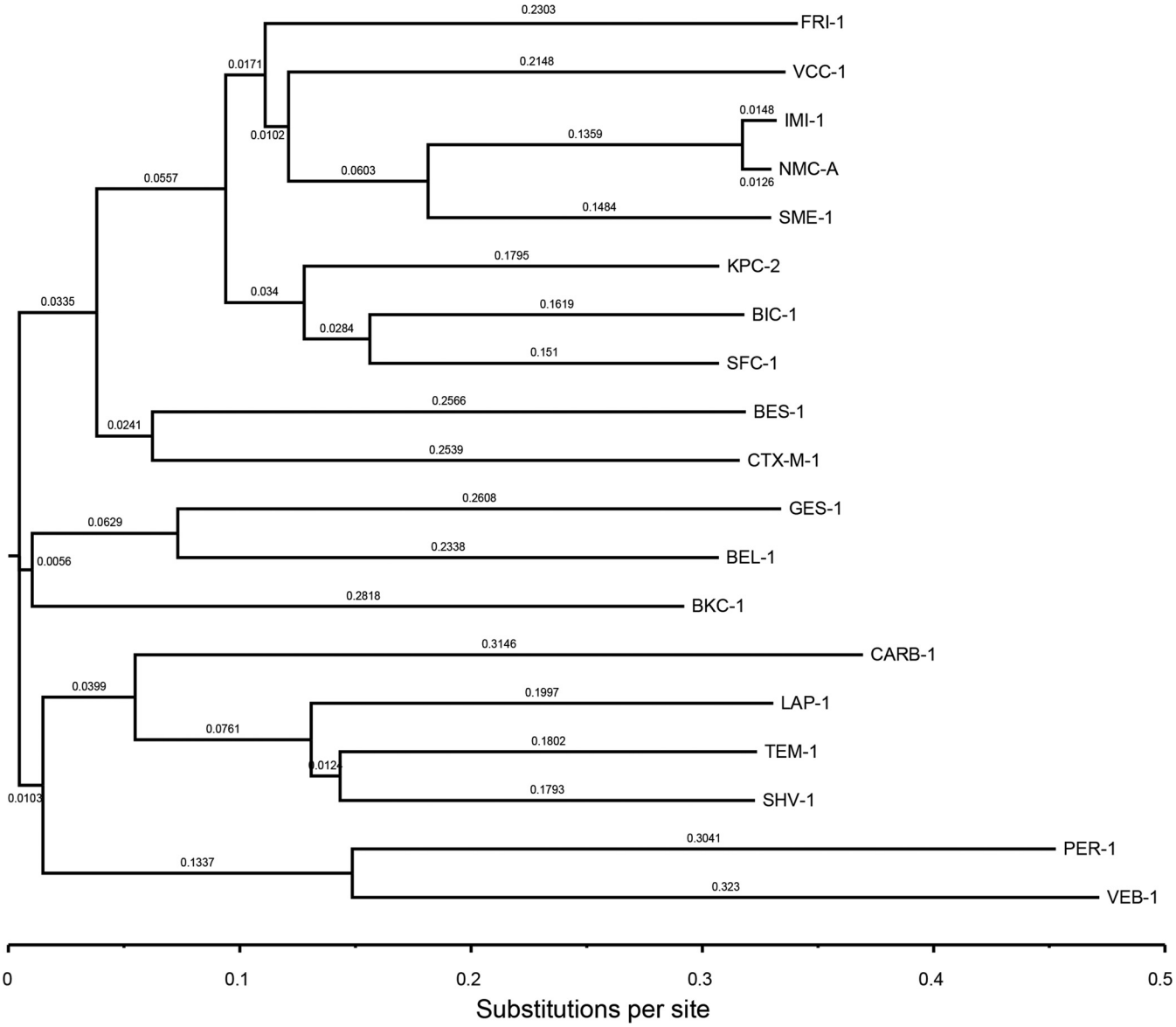

FIG 2 Dendrogram of 18 representative class A β-lactamases using precursor amino acid sequences. The alignment and neighbor-joining tree were produced using the Clustal Omega program from EMBL-EBI bioinformatics services (http://www.ebi.ac.uk/Tools/msa/clustalo/), and the tree was visualized using the FigTree (v1.4.2) viewer (http://tree.bio.ed.ac.uk/software/figtree/). Branch lengths are scaled to the number of amino acid changes per site. The β-lactamases (GenBank accession numbers) are as follows: BEL-1 (DQ089809), BES-1 (AF234999), BIC-1 (GQ260093), BKC-1 (KP689347), CARB-1 (D86225), CTX-M-1 (X92506), FRI-1 (KT192551) GES-1 (AF156486), IMI-1 (U50278), KPC-2 (AY034847), LAP-1 (EF026092), NMC-A (Z21956), PER-1 (Z21957), SFC-1 (AY354402), SHV-1 (AF148850), SME-1 (Z28968), TEM-1 (AY458016), VEB-1 (AF010416), and VCC-1 (KT818596).

carbapenems (37). In summation, the biochemical and genetic data were consistent with a carbapenemase function for VCC-1.

The bla_{VCC-1} sequence was present in two copies on the chromosome and was associated with three copies of the insertion element IS*Vch9* in a novel 8.5-kb region that may have been acquired by transposition from an unknown source (Fig. 1). Direct transfer of bla_{VCC-1} to *E. coli* could not be demonstrated *in vitro*, but it remains to be seen if dissemination in the environment or a clinical setting could occur. The bla_{VCC-1} region was inserted into the end of the *vrgG* gene, which encodes an effector protein of the type VI secretion system (38). Thus, in *V. cholerae* N14-02106, the VgrG protein would be a hybrid, with the last 20 amino acids being encoded by IS*Vch9*. If the VrgG protein is produced, it would be interesting to know if the virulence of *V. cholerae* N14-02106 is negatively affected by chimeric VrgG. The presence of VCC-1 in a *V. cholerae* isolate found in frozen shrimp could result in its distribution through the food chain across wide geographical areas and population groups. *V. cholerae* N14-02106 is not multidrug resistant, so there are options for the treatment of an infection with this organism, possibly with 3rd- or 4th-generation cephalosporins. The isolate was from a farmed shrimp from India, where limited control of antimicrobial use encourages the emergence of multidrug-resistant organisms both in the environment and in health care settings (39).

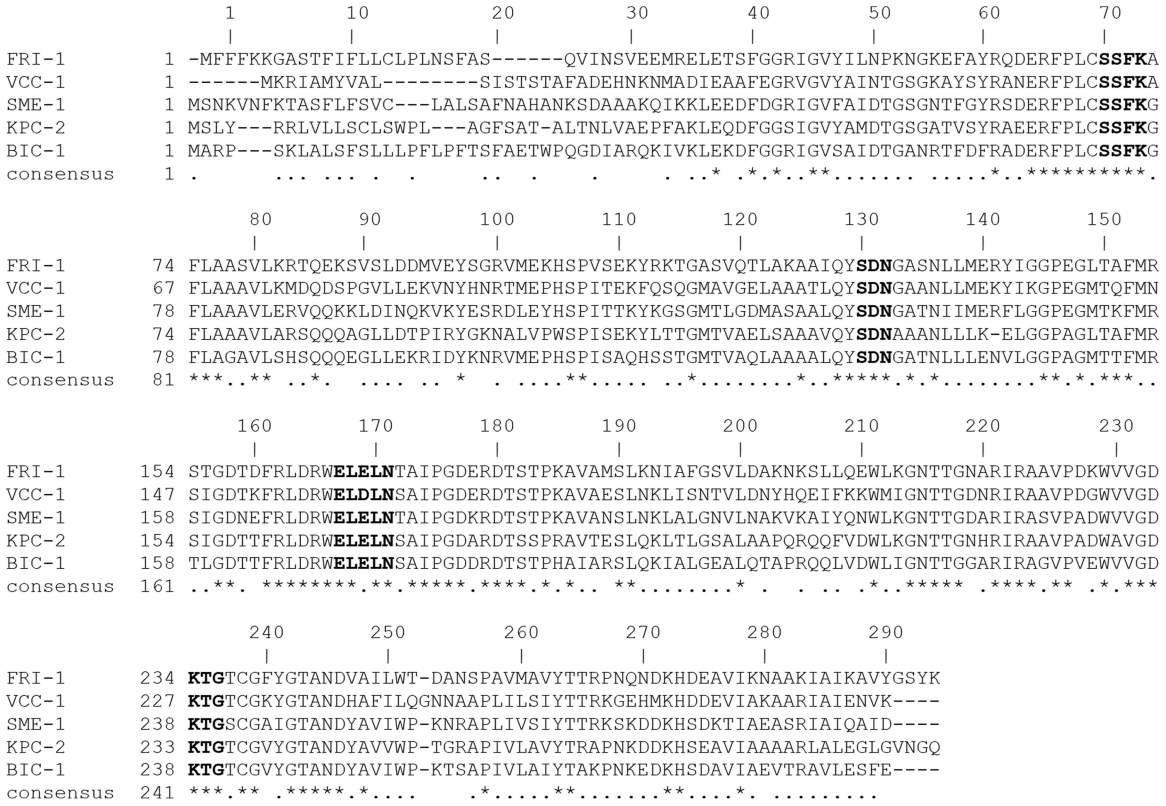

FIG 3 Amino acid sequence alignment of VCC-1 with the sequences of previously described carbapenemases. Signature motifs of β-lactamases are indicated in bold. The sequence numbering follows the Ambler scheme for class A β-lactamases. GenBank accession numbers are listed in the legend to Fig. 2.

Expression of VCC in *E. coli* TOP10 cells via pVCC-1 did not impart the same high-level carbapenem resistance seen with N14-02106; similar MIC differences were seen in the initial characterization of bla_{FRI}, bla_{BIC}, bla_{BKC}, bla_{SFC}, bla_{IMI}, and bla_{KPC} (32–36, 40). In the case of bla_{KPC}, cells of the clinical isolate contained a permeability defect that contributed to the elevated MICs of cell wall-active antibiotics. It is possible that *V. cholerae* N14-02106 cells have lower permeability than *E. coli* TOP10 cells, resulting in a higher carbapenem MIC; however, the cephalosporins MICs were lower in N14-02106, indicating that cell permeability was at least not the sole determinant of the differences in the MICs. A number of factors could have contributed to the change in MIC when a VCC was moved from a member of the *Vibrionaceae* to a member of the *Enterobacteriaceae*, including differences in gene expression and engagement of secretory machinery. Furthermore, previous work has shown that the complement of penicillin binding proteins and their specific affinities for cell wall-active antibi-

TABLE 1 MICs of β-lactams for *V. cholerae* N14-02106, *E. coli* TOP10 harboring recombinant plasmid pVCC-1, and the *E. coli* TOP10 parent strain

β-Lactam	MIC (μg/ml)[a]		
	V. cholerae N14-02106	*E. coli* TOP10(pVCC-1)[b]	*E. coli* TOP10
Ceftriaxone	0.39	1.5	0.064
Cefotaxime	0.064	0.38	0.094
Ceftazidime	0.75	3	0.75
Cefoxitin	16	12	6
Cefepime	1.5	0.5	0.094
Aztreonam	>256	48	0.094
Amoxicillin + CLA[c]	16	64	4
Piperacillin + TZB[d]	48	32	1.5
Imipenem	>32	12	0.5
Meropenem	>32	2	0.047
Ertapenem	>32	1.5	0.004
Doripenem	>32	2	0.032

[a] MICs were determined with Etest gradient strips.
[b] pVCC-1 contains bla_{VCC-1} cloned into pCR-XL-TOPO.
[c] CLA, clavulanic acid at a fixed concentration of 4 μg/ml.
[d] TZB, tazobactam at a fixed concentration of 4 μg/ml.

TABLE 2 Kinetic parameters of VCC-1

Substrate	k_{cat} (s^{-1})[a]	K_m (μM)[a]	k_{cat}/K_m ($\mu M^{-1}\ s^{-1}$)
Benzylpenicillin	37.8	136.0	0.278
Oxacillin	153.3	170.2	0.901
Cephalothin	320.1	593.0	0.540
Cefoxitin	>0.8[b]	>1,000	ND[c]
Cefotaxime	>4.5[b]	>1,000	ND
Ceftazidime	>0.2[b]	>1,000	ND
Aztreonam	>44.6[b]	>1,000	ND
Imipenem	190.2	876.8	0.217
Meropenem	11.2	70.1	0.160

[a] k_{cat} and K_m were estimated by fitting initial rates to the Michaelis-Menten equation using nonlinear regression. Values represent the averages from duplicates, where the range/mean was <10% in all cases.
[b] k_{cat} not determinable due to high K_m; activity at 1,000 μM is reported.
[c] ND, not determinable due to a high K_m.

otics and β-lactamase inhibitors contributed to differences in β-lactam susceptibility (41–44).

In conclusion, this is the first characterization of a class A carbapenemase found in a member of the *Vibrionaceae* and the first report of a novel class A carbapenemase discovered in food. Since the 1990s, we have seen the emergence of Ambler class A carbapenemases, which are usually first isolated from clinical samples. In this report, we describe one of the few instances where a new carbapenemase was discovered from a nonclinical and nonenteric source, the other example being BIC-1, which was found in a pseudomonad isolated from the Seine River (33). The discovery of VCC-1 was directly facilitated by the use of whole-genome sequencing, which is the first use of this technique to discover an enzyme of this class. Finally, this work highlights the need for inclusion of food and, possibly, environmental sources in antimicrobial resistance surveillance, as we will likely see an increase in the diversity of carbapenemases. Understanding and tracking of the potential spread from the environment into the clinic will be important for future infection control measures.

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