Carbapenemase VCC-1–Producing *Vibrio cholerae* in Coastal Waters of Germany

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During antimicrobial drug resistance testing for *Vibrio* spp. from coastal waters of Germany, we identified 4 nontoxigenic, carbapenem-resistant *V. cholerae* isolates. We used whole-genome sequencing to identify the carbapenemase gene bla_{VCC-1}. In addition, a molecular survey showed that more bla_{VCC-1}–harboring isolates are present in coastal waters of Germany.

Mangat et al. recently identified a novel ambler class A carbapenemase (VCC-1) in nontoxigenic *Vibrio cholerae* isolated from imported retail shrimp from India intended for human consumption in Canada (*1*). Occurrence of bla_{VCC-1}–harboring bacteria in seafood might be caused by uptake of *V. cholerae* in the aquatic environment. Lutz et al. reported worldwide distribution of *V. cholerae* non–O1/O139 strains in coastal waters with low salinity (*2*). Some of these strains were associated with wound infections and with diarrheal diseases after ingestion of contaminated seafood (*3*,*4*).

An antimicrobial resistance survey of potentially pathogenic *Vibrio* spp. recovered from coastal waters of Germany identified 4 carbapenem-resistant *V. cholerae* non–O1/O139 isolates (*5*). These isolates were detected in the Baltic Sea (VN-2997, Eckernförde) and North Sea (VN-2808, Büsum; VN-2825, Speicherkoog; VN-2923, unknown). We used whole-genome sequencing to examine the genetic basis of carbapenem resistance in these strains.

We isolated genomic DNA by using the Easy-DNA Kit (Invitrogen, Carlsbad, CA, USA). This DNA was used for preparation of libraries by using the Nextera-XT-DNA Sample Preparation Kit (Illumina Inc., San Diego, CA, USA) and sequenced by using an MiSeq-benchtop-sequencer, MiSeq-Reagent version 2 (300 cycles), and two 150-bp paired-end reads (Illumina Inc.). We then performed de novo assemblies of reads by using SPAdes version 3.5.0 (http://spades.bioinf.spbau.ru/release3.5.0/manual.html). We deposited sequences in GenBank and performed genome annotation by using the NCBI Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/).

We found bla_{VCC-1} in all isolates on contigs of 2,135 bp (VN-2825, VN-2997), 2,139 bp (VN-2923), and 2,737 bp (VN-2808). The bla_{VCC-1}-coding sequences and flanking nucleotide sequences were 100% identical among the strains, as determined by sequence alignments. We also identified the main characteristics of *V. cholerae* genomes (Table; online Technical Appendix Figure, https://wwwnc.cdc.gov/EID/article/23/10/16-1625-Techapp1.pdf). Overall, the genomes belong to the same multilocus sequence type (ST), ST336 (*adk* 57, *gyrB* 76, *mdh* 14, *metE* 115, *pntA* 18, *purM* 1, *pyrC* 101) (*6*).

We performed functional studies of the entire bla_{VCC-1}–harboring region (pVCC-1C, 2.7 kb) and bla_{VCC-1} gene (pVCC-1G, 0.9 kb) of *V. cholerae* VN-2808 (online Technical Appendix Figure) by molecular cloning of PCR-amplified regions according to standard procedures (*7*). After transformation of *Escherichia coli* GeneHogs (Invitrogen, Darmstadt, Germany) and susceptibility testing for aztreonam, imipenem, and meropenem as described (*5*), both constructs showed decreased inhibition zone diameters compared with that for *E. coli* GeneHogs vector. We observed slightly reduced drug susceptibility levels or intermediate resistance levels, as observed in *V. cholerae* VN-2808 (online Technical Appendix Figure).

On the basis of these results, we conducted bla_{VCC-1} screening of the *V. cholerae* collection at the German Federal Institute for Risk Assessment (Berlin, Germany). This collection contains 312 toxigenic and nontoxigenic isolates from human, environmental, and food origin obtained in Europe (n = 218), Africa (n = 20), Asia (n = 18), North America (n = 1), South America (n =1), and of unknown origin (n = 54) during 1941–2015.

We performed PCR by using primers (bla_{VCC-1}-forward/reverse: 5′-ATCTCTACTTCAACAGCTTTCG/CCTAGCTGCTTTAGCAATCAC-3′) with denaturation at 94°C for 120 s; 35 cycles of denaturation at 94°C for 15 s, annealing at 53°C for 30 s, and elongation for 210 s at 72°C; and a final elongation at 72°C for 1 min. This testing detected bla_{VCC-1} in 3 (1.6%) *ctx*-negative, non–O1/O139 *V. cholerae* isolates obtained from waters of the port of Husum, Germany, on the North Sea during 2015. Sanger sequencing confirmed the presence of bla_{VCC-1}. These 3 isolates belong to the new multilocus ST516. This type is divergent from ST336 only for the novel *pyrC* 150 variant, which was recently deposited in the PubMLST database (https://pubmlst.org).

In conclusion, this study showed the presence of 7 VCC-1 carbapenemase-producing *V. cholerae* at different locations on the coastline of Germany. The bla_{VCC-1} flanking

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Table. Genome characteristics of 4 carbapenemase-producing *Vibrio cholerae* isolates from coastal waters of Germany*

Characteristic	Isolate			
	VN-2808	VN-2825	VN-2923	VN-2997
No. genes	3,934	3,921	4,040	3,933
Coding genes	3,545	3,608	3,650	3,612
No. CDS	3,813	3,803	3,920	3,814
Coding CDS	3,545	3,608	3,650	3,612
No. RNA genes	121	118	120	119
No. rRNAs (5S, 16S, 23S)	10, 12, 7	10, 12, 4	10, 11, 7	10, 12, 4
Complete rRNAs (5S, 23S)	10, 1	10, 1	10, 1	10, 1
Partial rRNAs (16S, 23S)	12, 6	12, 3	11, 6	12, 3
No. tRNAs	88	88	88	89
No. noncoding RNAs	4	4	4	4
No. pseudogenes	268	195	270	202
Ambiguous residues	0	0	0	0
Frameshifted	39	37	40	37
Incomplete	220	144	221	152
Internal stop	20	25	22	23
Multiple problems	11	11	13	10
No. predicted prophages	5	2	5	2
Intact	2	0	1	0
Incomplete	3	1	4	2
Questionable	0	1	0	0
No. plasmids	ND	ND	ND	ND
Antibiotic resistance				
β-lactam†	*bla*$_{VCC-1}$ (100%)	*bla*$_{VCC-1}$ (100%)	*bla*$_{VCC-1}$ (100%)	*bla*$_{VCC-1}$ (100%)
MLST type	ST336	ST336	ST336	ST336
GenBank information				
Bioproject no.	PRJNA331077	PRJNA331078	PRJNA331079	PRJNA331080
Biosample no.	SAMN05437226	SAMN0537225	SAMN0537224	SAMN0537223
Accession no.	MCBB00000000	MCBA00000000	MCAZ00000000	MCAY00000000

*In silico analyses were conducted by using the services of the NCB Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/), the Center for Genomic Epidemiology (http://.genomicepidemiology.org), and PHAST (http://www.phast.wishartlab.com/). CDS, coding DNA sequence; MLST, multilocus sequencing typing; ND, not determined; ST, sequence type.
†Percentage values indicate level of nucleotide similarity against the reference gene (GenBank accession no. KT818596).

genetic sequences were identical in the 4 sequenced *V. cholerae* from Germany and appeared to be different from the strain isolated in Canada, which probably originated in India (online Technical Appendix Figure). This finding suggests that *bla*$_{VCC-1}$ was acquired by *V. cholerae* from a yet unknown progenitor on at least 2 occasions. Strains from Germany probably belong to the autochthonous microflora and represent an environmental reservoir of carbapenem resistance in coastal waters. *bla*$_{VCC-1}$–encoding *V. cholerae* might be taken up by mussels, shrimps, and fish and then enter the food chain.

Because carbapenems are needed for treatment of severe infections with multidrug-resistant bacteria in humans, the presence of bacteria with acquired, and thereby potentially transferable, carbapenemases in environments near human activities is a major public health concern (*8*). To date, acquired carbapenemases were detected mainly in clinical isolates and only rarely in environmental and foodborne bacteria (*9*,*10*). Exposure of humans to carbapenemase-producing pathogenic bacteria by ingestion of contaminated food products or by direct contact with contaminated water might pose a threat to public health. Our findings indicate that surveillance for antimicrobial drug resistance should be extended to locations of human activities and foods of aquatic origin.

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References

1. Mangat CS, Boyd D, Janecko N, Martz SL, Desruisseau A, Carpenter M, et al. Characterization of VCC-1, a novel ambler class A carbapenemase from *Vibrio cholerae* isolated from imported retail shrimp sold in Canada. Antimicrob Agents Chemother. 2016;60:1819–25. http://dx.doi.org/10.1128/AAC.02812-15
2. Lutz C, Erken M, Noorian P, Sun S, McDougald D. Environmental reservoirs and mechanisms of persistence of *Vibrio cholerae.* Front Microbiol. 2013;4:375. http://dx.doi.org/10.3389/fmicb.2013.00375
3. Deshayes S, Daurel C, Cattoir V, Parienti JJ, Quilici ML, de La Blanchardière A. Non-O1, non-O139 *Vibrio cholerae* bacteraemia:

case report and literature review. Springerplus. 2015;4:575. http://dx.doi.org/10.1186/s40064-015-1346-3
4. Engel MF, Muijsken MA, Mooi-Kokenberg E, Kuijper EJ, van Westerloo DJ. *Vibrio cholerae* non-O1 bacteraemia: description of three cases in the Netherlands and a literature review. Euro Surveill. 2016;21:30197. http://dx.doi.org/10.2807/1560-7917.ES.2016.21.15.30197
5. Bier N, Schwartz K, Guerra B, Strauch E. Survey on antimicrobial resistance patterns in *Vibrio vulnificus* and *Vibrio cholerae* non-O1/non-O139 in Germany reveals carbapenemase-producing *Vibrio cholerae* in coastal waters. Front Microbiol. 2015;6:1179. http://dx.doi.org/10.3389/fmicb.2015.01179
6. Jolley KA, Maiden MC. BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics. 2010;11:595. http://dx.doi.org/10.1186/1471-2105-11-595
7. Sambrook JF, Russell DW. Molecular cloning: a laboratory manual. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2001.
8. EFSA Panel on Biological Hazards (BIOHAZ). Scientific opinion on carbapenem resistance in food animal ecosystems. EFSA Journal. 2013;11:3501 [cited 2017 Aug 2]. http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3501/pdf
9. Jean SS, Lee WS, Lam C, Hsu CW, Chen RJ, Hsueh PR. Carbapenemase-producing gram-negative bacteria: current epidemics, antimicrobial susceptibility and treatment options. Future Microbiol. 2015;10:407–25. http://dx.doi.org/10.2217/fmb.14.135
10. Woodford N, Wareham DW, Guerra B, Teale C. Carbapenemase-producing *Enterobacteriaceae* and non-*Enterobacteriaceae* from animals and the environment: an emerging public health risk of our own making? J Antimicrob Chemother. 2014;69:287–91. http://dx.doi.org/10.1093/jac/dkt392

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Autochthonous Transmission of East/Central/South African Genotype Chikungunya Virus, Brazil

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We isolated East/Central/South African genotype chikungunya virus during the 2016 epidemic in Rio de Janeiro, Brazil. Genome sequencing revealed unique mutations in the nonstructural protein 4 (NSP4-A481D) and envelope protein 1 (E1-K211T). Moreover, all Brazil East/Central/South isolates shared the exclusive mutations E1-M407L and E2-A103T.

Chikungunya virus (CHIKV) is an alphavirus (family *Togaviridae*) transmitted by *Aedes aegypti* and *Ae. albopictus* mosquitoes. Chikungunya fever is characterized by fever, intense polyarthralgia, headache, joint swelling, and rash. Polyarthralgia can persist for several months after the acute phase (*1*). The 3 main CHIKV genotypes are Asian, West African, and East/Central/South African (ECSA), in addition to the ECSA-derived Indian Ocean lineage (*1*). Genetic analysis of CHIKV genomes has shown substitutions in envelope (E) 1 and E2 proteins that affect virus adaptability to *Aedes* mosquitoes. For example, E1-K211E and E2-V264A mutations have been reported to increase CHIKV fitness in *Ae. aegypti* (*2*), whereas E1-A226V and E2-L210Q mutations have been associated with improved adaptability to *Ae. albopictus* mosquitoes (*3,4*). The E1-T98A mutation enhances the vector-adaptability effect of E1-A226V, which is otherwise restricted by epistatic interactions between E1-98T and E1-A226V (*4*).

Autochthonous transmission of CHIKV (Asian genotype) in Brazil first occurred in 2014 in Oiapoque, Brazilian Amazon, 1 year after CHIKV introduction in the Caribbean (*1*). Since late 2014, autochthonous cases of the ECSA genotype have been reported in northeastern Brazil (*1*), a region of sustained cocirculation of dengue virus (DENV) for decades and the epicenter of recent Zika virus outbreaks (*5*).

Until late 2015, only a few imported cases of CHIKV (Asian genotype) had been reported in Rio de Janeiro (*6*). However, in December 2015, ten autochthonous CHIKV transmissions were reported, followed by an increase to 11,602 by August 2016, which accounted for 88.9% of reported cases in the state. Of these, 1,868 have been laboratory confirmed as CHIKV, leading to the highest incidence of CHIKV infection in southeastern Brazil (*7*). Nevertheless, the CHIKV genotype associated with the epidemic in Rio de Janeiro remains unknown.

On March 16, 2016, the emergency laboratory of the Brazilian Army Institute of Biology at Rio de Janeiro collected blood samples from a 16-year-old girl and a 29-year-old man who sought care at the associated military hospital. The patients had fever (40°C) for 24–48 h, debilitating polyarthralgia, and myalgia. The man also had exanthema and itching. The diagnostic hypotheses were Zika, dengue, or chikungunya, given their similar clinical symptoms (*5*). Laboratory findings were unremarkable except for

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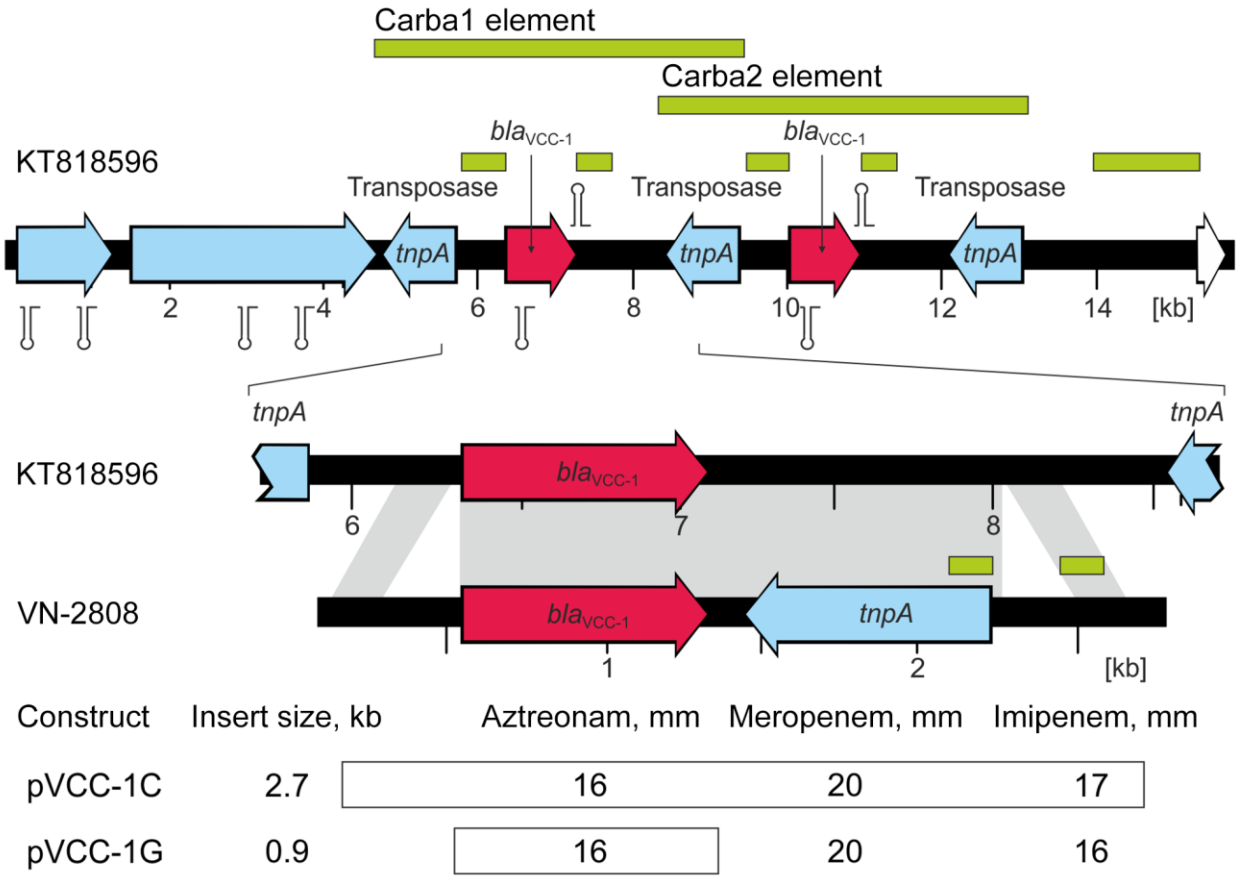
Technical Appendix



Construct	Insert size, kb	Aztreonam, mm	Meropenem, mm	Imipenem, mm
pVCC-1C	2.7	16	20	17
pVCC-1G	0.9	16	20	16

Technical Appendix Figure. Schematic organization of the *bla*$_{VCC-1}$–encoding region of carbapenemase VCC-1–producing *Vibrio cholerae* VN-2808 in coastal waters of Germany. Identical (100%) DNA sequences of the *bla*$_{VCC-1}$-encoding region of *V. cholerae* N14–02106 (KT818596) and VN-2808 are connected by gray shading. Repetitive sequences are indicated by green bars. Inhibition zone diameters for aztreonam, meropenem and imipenem susceptibility testing conferred by recombinant plasmids pVCC-1C and pVCC-1G in *Escherichia coli* GeneHogs are given. Recombinant plasmids were generated by insertion of PCR products in the multiple cloning site of the vector pIV2. For the *V. cholerae* wild-type strain VN-2808, inhibition zone diameters were 16 mm for aztreonam, 19 mm for meropenem, and 16 mm for imipenem. Carba, carbapenemase.