



Draft Genome Sequences of Seven *Vibrio cholerae* Isolates from Adult Patients in Qatar

Ameena Al Malki,^a Kyle D. Brumfield,^{b,c} [®]Clement K. M. Tsui,^{d,e,f} Anjana Anand,^a Shah M. Rashed,^b Emad Ibrahim,^{g,h} Hamad Al Shamari,^a Anwar Huq,^b Rita R. Colwell,^{b,c} [®]Rashmi Fotedar^a

Department of Genetic Engineering, Biotechnology Centre, Doha, Qatar
Maryland Pathogen Research Institute, University of Maryland, College Park, Maryland, USA
CUniversity of Maryland Institute for Advance Computer Studies, University of Maryland, College Park, Maryland, USA
Department of Pathology, Sidra Medicine, Doha, Qatar
Department of Pathology and Laboratory Medicine, Weill Cornell Medicine–Qatar, Doha, Qatar
fDivision of Infectious Diseases, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada
Department of Microbiology, Hamad Medical Corporation, Doha, Qatar
^hQatar University, Biomedical Research Centre, Doha, Qatar

ABSTRACT We report the draft genome sequences of seven *Vibrio cholerae* isolates from patients. Four isolates were profiled as multilocus sequence type 69, serogroup O1, a subset of seventh-pandemic El Tor clonal isolates. Presented here are genome assemblies and evidence for major pathogenicity islands, virulence factors, and antimicrobial resistance genes.

Cholera is an acute diarrheal disease and is transmitted via untreated water carrying the etiological agent *Vibrio cholerae*. Serogroups O1 and O139 are the causative agents of the ongoing pandemic, the seventh, and sporadic outbreaks globally (1). *V. cholerae* O1 isolates can be classified as the classical or El Tor biotype based on genotypic and phenotypic characteristics (2). Since the 19th century, seven cholera pandemics have been recorded, and *V. cholerae* O1 El Tor is the most common serogroup (1, 3). Although cholera is endemic across Africa and Asia, the disease causes a serious public health burden in many places. However, *V. cholerae* has not been reported in Qatar.

Here, we report draft genome assemblies of seven *V. cholerae* strains from adults with cholera-like symptoms at Hamad Medical Corporation, Doha, Qatar. Strain H08 was iso-lated from blood, and six isolates were from stool. Briefly, swabs were inoculated onto mSuperCARBA solid medium (CHROMagar, France) and incubated under aerobic conditions at $35 \pm 2^{\circ}$ C for 18 to 24 h, minimizing exposure to light. After incubation, blue colonies were confirmed using the matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) Biotyper system (Bruker, MA). Antimicrobial susceptibility was determined using the Phoenix system (Becton, Dickinson, NJ). MICs for antibiotics were determined according to the CLSI breakpoints for *Vibrio* spp. (4). All sequence type 69 (ST69) isolates showed some level of resistance to the commonly employed antibiotics (4). *Vibrio* cultures were maintained in Difco LB broth (Fisher Scientific, Hampton, NH) with aeration at 37° C.

Genomic DNA was extracted using a ZymoBIOMICS DNA miniprep kit (Zymo Research, CA), and the concentrations were determined using a Qubit 4.0 fluorometer (Thermo Fisher Scientific, Waltham, MA). DNA libraries were constructed using the lonXpress Plus fragment library kit; they were enriched and barcoded using the lonXpress barcode adapter kit (Thermo Fisher, MA). PCR products were purified using SPRIselect reagent (Beckman Coulter, Indianapolis, IN). Sequencing was performed Anand A, Rashed SM, Ibrahim E, Al Shamari H, Huq A, Colwell RR, Fotedar R. 2021. Draft genome sequences of seven *Vibrio cholerae* isolates from adult patients in Qatar. Microbiol Resour Announc 10:e01489-20. https://doi.org/ 10.1128/MRA.01489-20.

Citation Al Malki A. Brumfield KD. Tsui CKM.

Editor Catherine Putonti, Loyola University Chicago

Copyright © 2021 Al Malki et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Rashmi Fotedar, rfotedar@hotmail.com.

Received 5 January 2021 Accepted 11 February 2021 Published 4 March 2021

				60	Δνα		No	Multilocus	
Isolate no./GenBank	No. of	Genome	N ₅₀	content	coverage	No. of	of	sequence	Predicted antimicrobial
accession no.	reads	size (bp)	(bp)	(%)	(×)	contigs	CDS^a	type	resistance genes (ResFinder)
H01/JACWAP000000000	4,174,356	4,034,608	194,613	47.53	176	80	3,629	1287	tet(34)
H03/JACWAQ000000000	3,715,840	4,040,646	122,900	47.47	129	99	3,633	69	tet(34), sul2, tet(59), dfrA1, catB9, aph(3")-Ib, aph(6)-Id
H06A/JACWAR000000000	7,778,339	4,041,525	170,527	47.47	348	75	3,629	69	tet(34), sul2, tet(59), dfrA1, catB9, aph(3")-Ib, aph(6)-Id
H08/JACWAS00000000	15,621,973	3,935,466	529,619	47.53	700	60	3,536	1296	tet(34)
H09/JACWAT000000000	5,559,609	4,042,871	141,878	47.47	192	102	3,646	69	tet(34), sul2, tet(59), dfrA1, catB9, aph(3")-Ib, aph(6)-Id
H10/JACWAU000000000	7,684,224	4,133,920	170,440	47.46	252	87	3,695	1301	tet(34)
H12/JACWAV000000000	18,701,382	4,027,045	180,171	47.51	900	84	3,620	69	tet(34), sul2, tet(59), dfrA1, catB9, aph(3")-lb, aph(6)-ld

TABLE 1 Summary of genome statistics and genetic mechanism of antibiotic resistance

^a CDS, coding DNA sequences.

on an Ion S5 XL semiconductor sequencer (Ion Torrent; Thermo Fisher Scientific) to generate 200-bp sequence reads. Adapter sequences were removed, and low-quality bases were trimmed using Trim Galore v.0.6.5 (http://www.bioinformatics.babraham.ac .uk/projects/trim_galore/). Read quality was confirmed using FastQC v.0.11.9 (https:// github.com/s-andrews/FastQC), and the reads were assembled using SPAdes v.3.9.0 (5) with the options "-careful," to reduce the number of misassembles, and "-cov-cutoff auto," to remove misassembled low-coverage contigs. Small contigs (<500 bp) were discarded. Assembly statistics were assessed using QUAST v.5.0.2 (6). Multilocus sequence types (MLST) and antimicrobial resistance genes were predicted using the mlst (https://github.com/tseemann/mlst) and ResFinder v.3.2 databases (7) through abricate v.0.9.8 (https://github.com/tseemann/abricate) based on ≥70% coverage and ≥90% sequence identity. Any unknown MLST sequences that did not match the existing alleles were submitted to pubMLST (https://pubmlst.org/ vcholerae/). Virulence genes, pathogenicity islands (8), and O1 and O139 antigenencoding genes were typed using CholeraeFinder v.1.0 (https://cge.cbs.dtu.dk/ services/CholeraeFinder-1.0/). The genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline v.4.11 (9). Default parameters were used for all software unless otherwise specified.

Genome statistics and information for the seven isolates are summarized in Table 1. Four isolates (H03, H06A, H09, and H12) were identified as ST69, possessing genomic islands (GI) VSP-1, VSP-2, VPI-1, and VPI-2, as well as toxin/virulence genes, including *toxR*, *tcpA*, *rtxA*, *hlyA*, and *ctxA*, characteristics of seventh-pandemic *V. cholerae* O1 El Tor (1). All of the O1 El Tor isolates also carried *sul2*, *tet*(59), *dfrA1*, *catB9*, *aph*(*3''*)-*lb*, and *aph*(*6*)-*ld*, which code for resistance to the antibiotics sulfamethoxazole, tetracycline, trimethoprim, chloramphenicol, and streptomycin. Isolates H01, H08, and H10, however, were profiled as non-O1/non-O139. These isolates provide baseline information on the diversity of *V. cholerae* in Qatar.

Data availability. The whole-genome shotgun data from this study have been deposited in the DDBJ/ENA/GenBank repositories under SRA number PRJNA656914. The genome assembly versions described in this paper are the first versions.

ACKNOWLEDGMENTS

This work was partially supported by a research grant (NPRP 8-1252-1-233) from the Qatar National Research Fund (a member of the Qatar Foundation) to Rashmi Fotedar (MME), Anwar Hug (UMD), and Rita R. Colwell (UMD).

We declare that we have no conflict of interest.

This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

- Hasan NA, Choi SY, Eppinger M, Clark PW, Chen A, Alam M, Haley BJ, Taviani E, Hine E, Su Q, Tallon LJ, Prosper JB, Furth K, Hoq MM, Li H, Fraser-Liggett CM, Cravioto A, Huq A, Ravel J, Cebula TA, Colwell RR. 2012. Genomic diversity of 2010 Haitian cholera outbreak strains. Proc Natl Acad Sci U S A 109:E2010–E2017. https://doi.org/10.1073/pnas.1207359109.
- Brumfield KD, Carignan BM, Ray JN, Jumpre PE, Son MS. 2017. Laboratory techniques used to maintain and differentiate biotypes of *Vibrio cholerae* clinical and environmental isolates. J Vis Exp 2017(123):e55760. https://doi.org/10.3791/55760.
- Islam MT, Alam M, Boucher Y. 2017. Emergence, ecology and dispersal of the pandemic generating *Vibrio cholerae* lineage. Int Microbiol 20:106–115. https://doi.org/10.2436/20.1501.01.291.
- Clinical and Laboratory Standards Institute. 2010. Methods for antimicrobial dilution and disk susceptibility testing of testing of infrequently isolated or fastidious bacteria, 2nd ed. Approved guideline; CLSI document M45-A2. CLSI, Wayne, PA.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N,

Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.

- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https:// doi.org/10.1093/bioinformatics/btt086.
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10 .1093/jac/dks261.
- Greig DR, Schaefer U, Octavia S, Hunter E, Chattaway MA, Dallman TJ, Jenkins C. 2018. Evaluation of whole-genome sequencing for identification and typing of *Vibrio cholerae*. J Clin Microbiol 56:e00831-18. https://doi .org/10.1128/JCM.00831-18.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi .org/10.1093/nar/gkw569.