Draft Genome Sequences of Nine *Vibrio* sp. Isolates from across the United States Closely Related to *Vibrio cholerae*

Mohammad Tarequl Islam, Kevin Liang, Monica S. Im, Jonathan Winkjer, Shelby Busby, Cheryl L. Tarr, Yan Boucher

---

**ABSTRACT**

We are reporting whole-genome sequences of nine *Vibrio* sp. isolates closely related to the waterborne human pathogen *Vibrio cholerae*. These isolates were recovered from sources, including human samples, from different regions of the United States. Genome analysis suggests that this group of isolates represents a highly divergent basal *V. cholerae* lineage or a closely related novel species.

The genus *Vibrio* is one of the most diverse and ubiquitous groups of marine bacteria, including species with significant clinical importance, such as *Vibrio cholerae*, which is the causative agent of the pandemic diarrheal disease cholera (1). Surveillance conducted under the Cholera and Other *Vibrio* Illness Surveillance (COVIS) program ([https://www.cdc.gov/vibrio/surveillance.html](https://www.cdc.gov/vibrio/surveillance.html)) revealed nine isolates indistinguishable from *V. cholerae* by traditional phenotypic tests but phylogenetically and genotypically divergent from that species.

Cultivation and laboratory identification of these strains based on *rpoB* sequence determination and phylogenetic reconstruction were done following standard procedures (2). Genomic DNA was extracted from the isolates using an ArchivePure DNA cell/tissue kit (5 PRIME) according to the manufacturer’s instructions. Sequencing libraries were prepared from the genomic DNA using the Nextera XT DNA library preparation kit (Illumina, San Diego, CA, USA) and sequenced using Illumina MiSeq sequencing platforms (2 × 250-bp paired-end reads). Quality control and *de novo* assembly of the reads were done using default parameters in CLC Genomics workbench 7 (Qiagen). The average genome size was 3.9 Mbp with an average G+C content of 47.3%. Detailed isolate information is outlined in Table 1. Genome annotations were done using RAST 2.0 (3) and the Prokaryotic Genome Annotation Pipeline (PGAP) ([https://www.ncbi.nlm.nih.gov/genome/annotation_prok/](https://www.ncbi.nlm.nih.gov/genome/annotation_prok/)). Average nucleotide identity (ANI) and *in silico* DNA-DNA hybridization (dDDH) values in comparison to those of the reference strains were calculated using JSpecies v1.2.1 (4) and Genome-to-Genome Distance Calculator (GGDC), respectively. Whole-genome alignment was performed using Mugsy v1.2.3 (5) with default parameters, and a maximum likelihood tree was built from this alignment using RaxML v8 (6) under the GTR+GAMMA model with 1,000 bootstrap replicates. The core genome phylogenetic tree and strains used for dDDH and ANI have been deposited in publicly available databases.

Species delineation values for prokaryotes have been considered 95% or higher for ANI and 70% or higher for dDDH (7). The nine sequenced strains shared 96.5% to 99% ANI and 82% to 85% dDDH values. On the other hand, in comparisons with representative *V. cholerae* strains, ANI ranged from 94% to 96% and dDDH from 65% to 70%. Core genome phylogeny shows that these strains form a strongly supported monophyletic clade basal to *V. cholerae*. Taken together, the tree topology and ANI and
dDDH values suggest that these nine isolates could belong to a new species representing the closest relative of *V. cholerae* known to date or a highly divergent lineage within the species.

All nine strains contain the toxin transcriptional regulator gene (*toxR*), which is the master regulator of virulence genes in *V. cholerae*, but lack major virulence factor and related genetic elements and/or genes typical of toxigenic *V. cholerae*, such as *ctxAB*, *tcpA*, and the two *Vibrio* pathogenicity islands VPI-1 and VPI-2. However, eight out of nine strains possessed RTX toxin (8), and two strains possessed genes for the type III secretion system, which is an established virulence factor for *V. cholerae* (9). The presence of these virulence-related genes, in addition to their isolation from clinical cases, underscores the pathogenetic potential of this monophyletic group of strains.

**Data availability.** The complete genome sequences were deposited in DDBJ/GenBank and raw reads were deposited in the SRA depository under the accession numbers listed in Table 1. Phylogenetic tree, ANI, and dDDH values were deposited in a publicly available repository under the DOI 10.7939/DVN/OZHLC2 (https://doi.org/10.7939/DVN/OZHLC2).

**ACKNOWLEDGMENTS**

This work is supported by the Natural Sciences and Engineering Research Council of Canada, the Integrated Microbial Biodiversity program of the Canadian Institute for Advanced Research (to Y.B.), and federal appropriations to the Centers for Disease Control and Prevention through the Advanced Molecular Detection Initiative.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

**REFERENCES**

1. Takemura AF, Chien DM, Polz MF. 2014. Associations and dynamics of Vibrionaceae in the environment, from the genus to the population level. Front Microbiol 5:38. https://doi.org/10.3389/fmicb.2014.00038.
2. Tarr CL, Patel JS, Puhr ND, Sowers EG, Bopp CA, Strockbine NA. 2007. Identification of *Vibrio* isolates by a multiplex PCR assay and *rpoB* sequence determination. J Clin Microbiol 45:134–140. https://doi.org/10.1128/JCM.01544-06.
3. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
4. Richter M, Rosselló-Móra R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A 106:19126–19131. https://doi.org/10.1073/pnas.0904091102.
5. Islam et al. 2014. Associations and dynamics of Vibrionaceae in the environment, from the genus to the population level. Front Microbiol 5:38. https://doi.org/10.3389/fmicb.2014.00038.
6. Richter M, Rosselló-Móra R, 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A 106:19126–19131. https://doi.org/10.1073/pnas.0904091102.
7. Lin W, Fullner KJ, Clayton R, Sexton JA, Rogers MB, Calia KE, Calderwood SB, Fraser C, Mekalanos JJ. 1999. Identification of a Vibrio cholerae RTX toxin gene cluster that is tightly linked to the cholera toxin prophage. Proc Natl Acad Sci USA 96:1071–1076. https://doi.org/10.1073/pnas.9904612106.
8. Lin W, Fullner KJ, Clayton R, Sexton JA, Rogers MB, Calia KE, Calderwood SB, Fraser C, Mekalanos JJ. 1999. Identification of a Vibrio cholerae RTX toxin gene cluster that is tightly linked to the cholera toxin prophage. Proc Natl Acad Sci USA 96:1071–1076. https://doi.org/10.1073/pnas.9904612106.
9. Dziejman M, Serruto D, Tam VC, Sturtevant D, Direrhat P, Faruque SM, Rahman MH, Heidelberg JF, Deckr J, Li L, Montgomery KT, Grills G, Kucherlapati R, Mekalanos J. 2005. Genomic characterization of non-O1, non-O139 Vibrio cholerae reveals genes for a type III secretion system. Proc Natl Acad Sci U S A 102:3465–3470. https://doi.org/10.1073/pnas.0409918102.