





Insights into the Draft Genome Sequence of a Haitian Variant Vibrio cholerae Strain Isolated from a Clinical Setting in Kerala, South India

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ABSTRACT We report here the draft genome sequence of a Haitian variant *Vibrio cholerae* strain, W4-13, isolated from Kerala, South India, possessing cholera toxin gene in chromosomes I and II. The sequence will be useful to achieve a profound understanding on its evolution, with emphasis on its pathogenesis and antibiotic resistance.

India, being an area in which cholera is endemic, experiences periodic outbreaks of the disease even today. The appearance of evolved strains of *Vibrio cholerae*, the causative agent of cholera, and its dissemination are causes of great concern to all developing countries (1, 2). Major genetic traits of *V. cholerae* O1 Haitian isolates were previously reported (3).

The sequenced strain was isolated during a sporadic cholera outbreak in the Wayanad district of Kerala, South India, in 2013 from the stool sample of a diarrheal patient. The isolate was identified to be toxigenic, possessing all major virulence genes. The ctxB gene sequence was similar to that of the Haitian outbreak strains (ctxBT), and rstB had a genotype similar to those of the Kolkata variants. PCR analysis revealed that the strain possessed the cholera toxin gene in both chromosomes, unlike the Haitian variants reported so far. The isolate W4-13 was found to be resistant to trimethoprim, co-trimoxazole, streptomycin, and nalidixic acid by a disk diffusion assay. It also amplified the strB and sul2 genes responsible for streptomycin and sulfamethoxazole, respectively. The above-mentioned features prompted us to sequence the strain to achieve a profound understanding on its evolution, with emphasis on its virulence and antibiotic resistance determinants.

Total genomic DNA was isolated using the Wizard genomic DNA purification kit (Promega), per the manufacturer's protocol. The library was prepared using the Ion PGM library kit and loaded onto a 318 Chip version 2 provided by Ion Torrent (Life Technologies, Inc.). Sequencing by the Ion Torrent PGM platform yielded a total of 2,139,628 reads ($40\times$ genome coverage). The draft genome was assembled using SPAdes version 3.1 (4), which resulted in 123 contigs, with a total sequence length of 3,993,976 bp and an N_{50} of 56,569 bp. The longest contig was 159,256 bp in size. The strain showed a G+C content of 47.52%. The genome was annotated using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (5) and analyzed using the Rapid Annotations using Subsystems Technology (RAST) server (6). Resistance genes were predicted by ResFinder version 2.1 (7).

The annotation process identified 3,828 genes, 3,763 coding sequences, 791 pseudogenes, 553 subsystems, and 65 RNAs, including 9 rRNAs, 52 tRNAs, and 4 noncoding RNAs (ncRNAs). Genomic analysis revealed the presence of cholera toxin genes on both

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chromosomes. ResFinder detected an aminoglycoside gene, *strB*, and a sulfonamide resistance gene, *sul2*, with 100% identity, and an aminoglycoside gene (*strA*), phenicol resistance genes (*floR* and *catB9*), and a trimethoprim resistance gene (*dfrA1*), with 99% identity. Genes encoding multidrug efflux pumps and mobile element proteins were also identified. A detailed comparison of this strain to the prototype *V. cholerae* strains and Haitian outbreak strains will be presented in a future publication. Genomic information gathered from the comparative analysis will provide us a better understanding of the pathogenesis and resistance determinants of the evolved *V. cholerae* strains.

Accession number(s). The whole-genome shotgun project of the strain has been deposited at DDBJ/EMBL/GenBank under the accession number NIWX00000000. The version described in this paper is the first version, NIWX01000000.

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REFERENCES

- Chin CS, Sorenson J, Harris JB, Robins WP, Charles RC, Jean-Charles RR, Bullard J, Webster DR, Kasarskis A, Peluso P, Paxinos EE, Yamaichi Y, Calderwood SB, Mekalanos JJ, Schadt EE, Waldor MK. 2011. The origin of the Haitian cholera outbreak strain. N Engl J Med 364:33–42. https://doi. org/10.1056/NEJMoa1012928.
- Divya MP, Sivakumar KC, Sarada Devi KL, Remadevi S, Thomas S. 2014. Novel multiple mutations in the topoisomerase gene of Haitian variant Vibrio cholerae O1. Antimicrob Agents Chemother 58:4982–4983. https://doi.org/10.1128/AAC.03189-14.
- 3. Ghosh P, Naha A, Pazhani GP, Ramamurthy T, Mukhopadhyay AK. 2014. Genetic traits of *Vibrio cholerae* O1 Haitian isolates that are absent in contemporary strains from Kolkata, India. PLoS One 9:e112973. https://doi.org/10.1371/journal.pone.0112973.
- 4. 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 as-

- sembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
- Klimke W, Agarwala R, Badretdin A, Chetvernin S, Ciufo S, Fedorov B, Kiryutin B, O'Neill K, Resch W, Resenchuk S, Schafer S, Tolstoy I, Tatusova T. 2009. The National Center for Biotechnology Information's Protein Clusters Database. Nucleic Acids Res 37:D216–D223. https://doi.org/10.1093/nar/qkn734.
- 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.
- 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.

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