

# Genome Sequences from a Reemergence of *Vibrio cholerae* in Haiti, 2022 Reveal Relatedness to Previously Circulating Strains

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**KEYWORDS** *Vibrio cholerae*, WASH, cholera, outbreak

**Published** 6 March 2023

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The authors declare no conflict of interest.

**Editor** Alexander J. McAdam, Boston Children's Hospital

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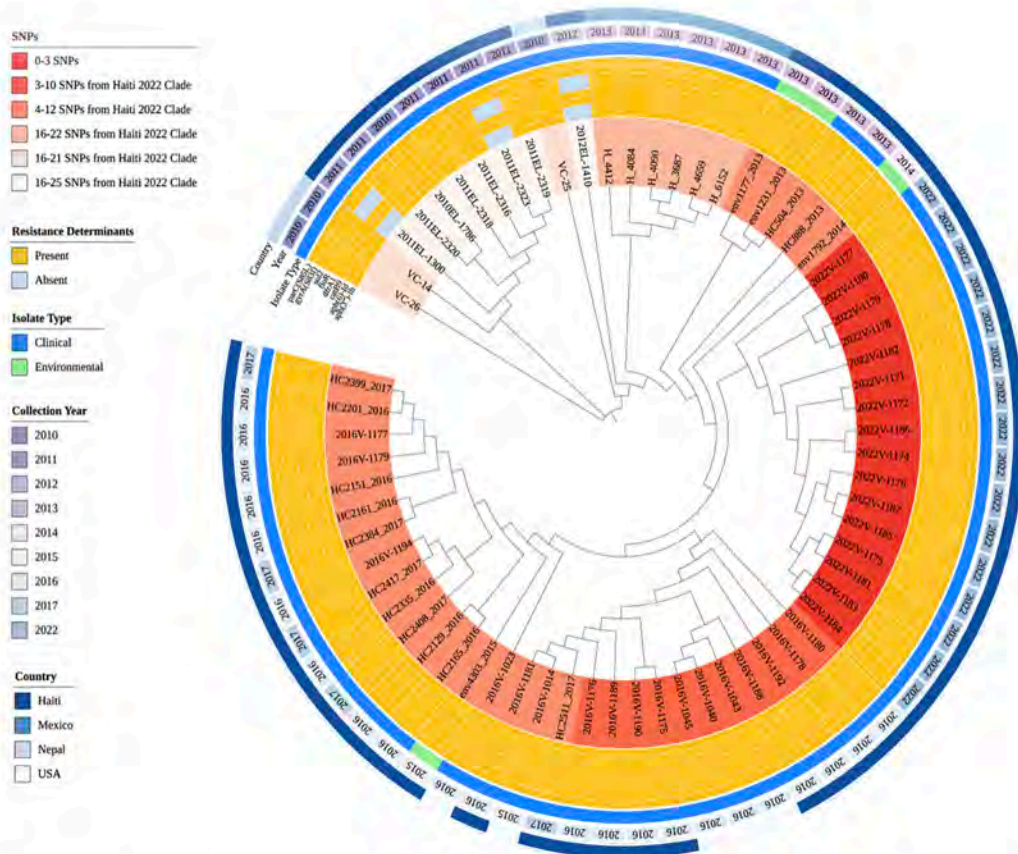
## LETTER

**A**fter more than 3 years without a documented cholera case, the Republic of Haiti reported its first resurgent case on 30 September 2022 (1–3). As of 18 February 2023, more than 27,000 cholera cases have been hospitalized and 594 deaths confirmed from all 10 departments (4). Here, we describe *Vibrio cholerae* isolates first characterized by the Laboratoire National de Santé Publique (LNSP) and include both genotypic and phenotypic antimicrobial resistance profiles. Whole-genome sequencing (WGS) analysis was compared with recently circulating cholera toxin-producing *V. cholerae* O1 in a maximum likelihood phylogeny.

LNSP sent 17 isolates collected from Centre, Grand-Anse, and Ouest departments between 30 September and 31 October 2022 to the Centers for Disease Control and Prevention (CDC) for species and toxin confirmation, antimicrobial susceptibility testing, and WGS analysis. CDC confirmed 16 isolates as toxigenic *V. cholerae* serogroup O1; 15 were serotype Ogawa, and one was Inaba. One isolate was confirmed as *Escherichia coli*. DNA libraries were prepared using Illumina reagents; sequencing was performed on the MiSeq and assessed for quality. Quality metrics for WGS analysis included a minimal Q-score of  $\geq 30$ , average *de novo* coverage of  $\geq 40\times$ , and genome length of  $\geq 4$  Mb. *De novo* assembly of 2010EL-1786 was performed using SPAdes v.3.14.0, and this strain served as the reference for a high-quality single nucleotide polymorphism (SNP) analysis using Lyve-SET (v1.1.4f). A phylogeny was visualized on iTOL.

Antimicrobial susceptibility testing by broth microdilution using CMV5AGNF panels (Sensititre, Westlake, OH) was performed according to the manufacturer's instructions and interpreted based on CLSI guidance (5). Reduced susceptibility to ciprofloxacin was defined as a MIC of  $\geq 0.25$   $\mu\text{g}/\text{mL}$  (6). Resistance determinants from sequences were found with the ResFinder database and by interrogating *gyrA* and *parC* genes (7). Isolates showed resistance to sulfisoxazole and trimethoprim-sulfamethoxazole, conferred by *sul2* and *dfrA1*, and reduced susceptibility to ciprofloxacin was attributed to *gyrA*(S83I) and *parC*(S85L) mutations. Susceptibility to azithromycin, chloramphenicol, and tetracycline was observed despite the detection of chloramphenicol resistance determinants (*catB9* and *floR*). Streptomycin resistance determinants were also observed [*aph(3'')-Ib* and *aph(6)-Id*]. Phenotypic and genotypic resistance findings are consistent with the Haiti 2010 outbreak strains (6, 8).

Haiti 2022 Outbreak genomes were compared with historical isolates from the CDC and genomes available on NCBI Pathogen Detection (9) closely related to the reference sequence 2010EL-1786 (Fig. 1) (8). All Haiti 2022 outbreak strains were very closely related to one another (0 to 3 SNPs apart). Furthermore, these strains were most closely related to 2016 isolates (3 to 10 SNPs apart) and other clinical and environmental isolates between 2013 and 2017 (4 to 12 SNPs apart) from Haiti. They were more distantly related to 2010–2012 Haiti isolates (16 to 25 SNPs apart) and 2013 isolates from Mexico (16 to 22 SNPs apart) previously characterized as imported from Haiti (10, 11). This analysis suggests a strong phylogenetic relationship to previously circulating *V. cholerae* in Haiti as opposed to an external introduction as the source of the outbreak. These data may be informative for characterizing potential origins of currently circulating strains, as there are multiple cholera outbreaks affecting thousands worldwide in regions that have been previously deemed cholera free for many years. Furthermore, this reemergence distinctly demonstrates the endemic potential of a strain that can cause multiple explosive cholera outbreaks over an extended period, as opposed to seasonal outbreaks caused by multiple sublineages in regions of hyperendemicity (12). A more detailed analysis that includes additional clinical and environmental isolates from Haiti and other regions may lend important insights into the evolutionary dynamics and selection of pandemic *V. cholerae*.



**FIG 1** A maximum likelihood phylogeny constructed using high-quality SNPs places the 16 confirmed toxigenic O1 Haiti 2022 samples in the context of other previous pandemic *Vibrio cholerae* isolates. An interactive version of this figure is available at <https://itol.embl.de/shared/atzbQKQc4aGB>.

**Data availability.** Sequence data are available through BioProject under accession no. [PRJNA266293](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA266293).

## ACKNOWLEDGMENTS

This project was supported in part by an appointment to the Research Participation Program at the Centers for Disease Control and Prevention administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the Centers for Disease Control and Prevention.

The findings and conclusions in this report are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention or by the U.S. Department of Health and Human Services.

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