



# New Variant of *Vibrio cholerae* O139 in Odisha, India

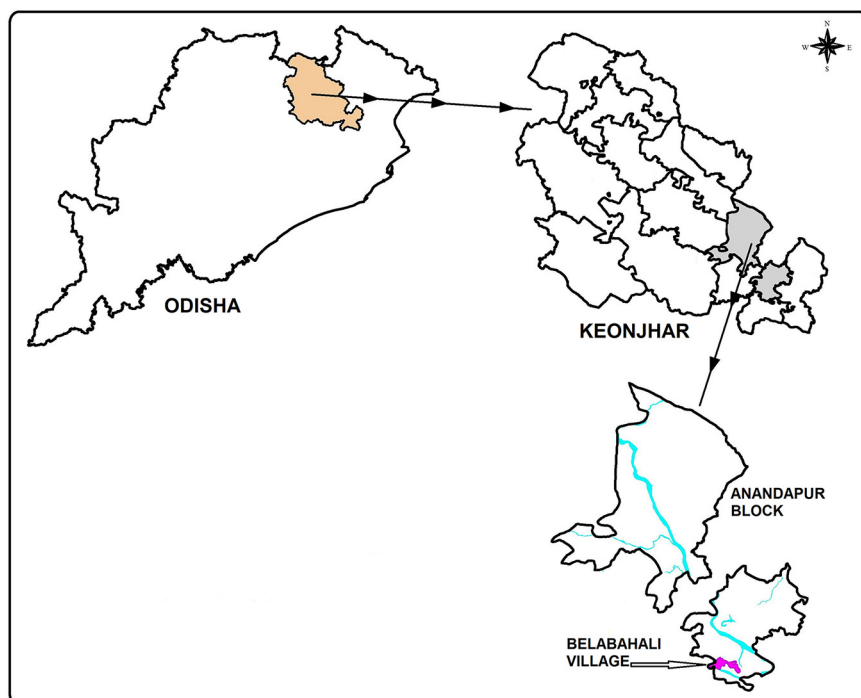
Bibhuti Bhusan Pal,<sup>a</sup> Anima Mohanty,<sup>a</sup> BhagyaLaxmi Biswal,<sup>a</sup> Smruti Ranjan Nayak<sup>a</sup>

<sup>a</sup>Microbiology Division, Regional Medical Research Centre (ICMR), Chandrasekharpur, Bhubaneswar, Odisha, India

**KEYWORDS** new variant, Odisha, *V. cholerae* O139

*Vibrio cholerae*, the causative agent of cholera, is endemic in many areas of the world. The *V. cholerae* serogroup O1, consisting of two biotypes (classical and El Tor), and the derivative serotype O139 can cause epidemics of cholera. *V. cholerae* O139 first emerged in South India during 1992, and subsequently in Bangladesh and China in 1993 (1). Three *V. cholerae* O139 isolates were reported from Bangladesh (2013 to 2014) (2), and 37 were reported from China (2013) (3). *V. cholerae* O139 isolates were reported for the first time in 1995 (4), and the last report was in 2006 (5), from Odisha. A large outbreak of severe watery diarrhea reported on 2 May 2017 from Belabahali village, Anandapur block, Keonjhar District, was investigated to find out the causative organism.

The village is located at the bank of Kosei River (Fig. 1), and the water supply of the village was directly through a borewell pipe submerged in the river. The index case was a 75-year-old female who was suffering from rice-watery stool, vomiting, abdominal cramping, and muscular pain on 30 April 2017. There was torrential rainfall for about 5 h on 1 May 2017. Suddenly, 18 severe watery diarrhea cases were reported on 2 May 2017; 67 diarrhea cases and no deaths were reported from 30 April 2017 to 11 May 2017. Twenty rectal swabs and 11 water samples, including the



**FIG 1** Map showing cholera outbreak village, Anandapur block, Keonjhar district.

**Citation** Pal BB, Mohanty A, Biswal B, Nayak SR. 2019. New variant of *Vibrio cholerae* O139 in Odisha, India. J Clin Microbiol 57:e01877-18. <https://doi.org/10.1128/JCM.01877-18>.

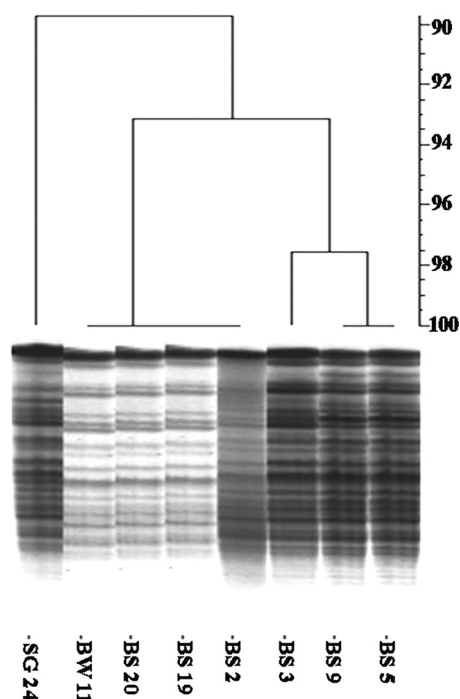
**Editor** Andrew B. Onderdonk, Brigham and Women's Hospital

**Copyright** © 2019 American Society for Microbiology. All Rights Reserved.

Address correspondence to Bibhuti Bhusan Pal, [bbpal\\_rmr@yahoo.co.in](mailto:bbpal_rmr@yahoo.co.in).

Accepted manuscript posted online 27 February 2019

Published 26 April 2019



**FIG 2** PFGE patterns of the NotI-digested *V. cholerae* O139 strains (clinical isolates BS2, BS3, BS5 BS9, BS19, and BS20; water isolate BW 11) from Odisha and the SG 24 (standard *V. cholerae* O139) strain, along with dendrogram analysis using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium). Analysis showed 3 distinct pulsotypes, suggesting 90% similarity with the standard strain.

starting supply water point, were processed per our previous practice (5). Fifteen (75%) out of 20 rectal swabs and one out of 11 water samples were positive for the *V. cholerae* O139 serogroup. *V. cholerae* O139 strains were resistant to ampicillin and streptomycin only. The selected *V. cholerae* O139 strains were subjected to double-mismatch-amplification mutation assay (DMAMA) PCR assay, sequencing, and pulsed-field gel electrophoresis (PFGE) analysis (6). The DMAMA PCR assay of the *ctxB* gene of all *V. cholerae* O139 isolates indicated that all were negative for *ctxB7* of Haitian variant *V. cholerae*; however, sequencing of the *ctxB* gene indicated that both the clinical and water isolates of *V. cholerae* O139 were prototype El Tor *ctxB* with a single mutation at position 132 of the amino acid sequence and the cysteine substituted by glutamine, therefore indicating a new genotype (GenBank accession number [MH423442](#)). The PFGE result for selected *V. cholerae* O139 strains, including the water isolate, indicated that there were three different pulsotypes (Fig. 2).

Torrential rainfall for 5 h on 1 May 2017 and a contaminated muddy water supply to the village were the major source of infection. The first emergence of *V. cholerae* O139 was reported in 1995 from Odisha (5). The *V. cholerae* O139 strains isolated in Bangladesh from 1993 to 2005 were 3 new *ctxB* genotypes, closely similar to *ctxB* El Tor (7), and were completely different from the present findings.

Three interesting findings emerged from this investigation, namely (i) the reemergence of *V. cholerae* O139 in Odisha after a hiatus of 10 years; (ii) the reemerged O139 strains carried a novel *ctxB* genotype, and this is the first report of such strains of O139 causing outbreaks of cholera; and (iii) a new variant of *V. cholerae* O139 has again emerged from the Bay of Bengal region (7). By sequencing and comparing hundreds of bacterial genomes, recent studies have shown that all of the explosive epidemics of cholera in Africa and the Americas in the past half-century arose after the arrival of new strains that had evolved in Asia (8, 9). Although the O139 serotype of *V. cholerae* has not spread to the African or American continents, the likelihood of this new variant spreading to other parts of the world should not be underestimated.

## ACKNOWLEDGMENTS

We thank S. Pati, Director, Regional Medical Research Centre (ICMR), Bhubaneswar, for her support; S. S. Bal, ADMO (PH), CDMO office, Keonjhar, for cooperation and sample collection; and G. B. Nair and T. Ramamurthy for their valuable suggestions. We also acknowledge the PFGE analysis rendered by D. V. Singh, Institute of Life Science, Bhubaneswar.

## REFERENCES

1. Li BS, Xiao Y, Wang DC, Tan HL, Ke BX, He DM, Ke CW, Zhang YH. 2016. Genetic relatedness of selected clinical *Vibrio cholerae* O139 isolates from the southern coastal area of China over a 20-year period. *Epidemiol Infect* 144:2679–2687. <https://doi.org/10.1017/S0950268816001059>.
2. Chowdhury F, Mather AE, Begum YA, Asaduzzaman M, Baby N, Sharmin S, Biswas R, Uddin M, LaRocque RC, Harris JB, Calderwood SB, Ryan ET, Clemens JD, Thomson NR, Qadri F. 2015. *Vibrio cholerae* serogroup O139: isolation from cholera patients and asymptomatic household family. *PLoS Negl Trop Dis* 9:e0004183. <https://doi.org/10.1371/journal.pntd.0004183>.
3. World Health Organization. 2014. Cholera annual report, 2013. *Wkly Epidemiol Rec* 89:345–356.
4. Pa BB, Khuntia HK, Anuradha A, Chhotray GP. 2000. Emergence of *Vibrio cholerae* O139 during 1995 in Orissa, India: a retrospective study. *Ind J Med Micro* 18:195–196.
5. Pal BB, Nayak SR, Khuntia HK. 2018. Epidemiology and antibiogram profile of *Vibrio cholerae* isolates between 2004–2013 from Odisha, India. *Jpn J Infect Dis* 71:99–103. <https://doi.org/10.7883/yoken.JJID.2017.193>.
6. Naha A, Pazhani GP, Ganguly M, Ghosh S, Ramamurthy T, Nandy RK, Nair GB, Takeda Y, Mukhopadhyay AK. 2012. Development and evaluation of a PCR assay for tracking the emergence and dissemination of Haitian variant *ctxB* in *Vibrio cholerae* O1 strains isolated from Kolkata, India. *JCM* 50:1733–1736. <https://doi.org/10.1128/JCM.00387-12>.
7. Bhuiyan NA, Nusrin S, Alam M, Morita M, Watanabe H, Ramamurthy T, Cravioto A, Nair GB. 2009. Changing genotypes of cholera toxin (CT) of *Vibrio cholerae* O139 in Bangladesh and description of three new CT genotypes. *FEMS Immunol Med Microbiol* 57:136–141. <https://doi.org/10.1111/j.1574-695X.2009.00590.x>.
8. Weill FX, Domman D, Njamkepo E, Tarr C, Rauzier J, Fawal N, Keddy KH, Salje H, Moore S, Mukhopadhyay AK, Bercion R, Luquero FJ, Ngandjio A, Dosso M, Monakhova E, Garin B, Bouchier C, Pazzani C, Mutreja A, Grunow R, Sidikou F, Bonte L, Breurec S, Damian M, Njanpop-Lafourcade BM, Sapriel G, Page AL, Hamze M, Henkens M, Chowdhury G, Mengel M, Koeck JL, Fournier JM, Dougan G, Grimont PAD, Parkhill J, Holt KE, Piarroux R, Ramamurthy T, Quilici ML, Thomson NR. 2017. Genomic history of the seventh pandemic of cholera in Africa. *Science* 358:785–789. <https://doi.org/10.1126/science.aad5901>.
9. Domman D, Quilici ML, Dorman MJ, Njamkepo E, Mutreja A, Mather AE, Delgado G, Morales-Espinosa R, Grimont PAD, Lizárraga-Partida ML, Bouchier C, Aanensen DM, Kuri-Morales P, Tarr CL, Dougan G, Parkhill J, Campos J, Cravioto A, Weill FX, Thomson NR. 2017. Integrated view of *Vibrio cholerae* in the Americas. *Science* 358:789–793. <https://doi.org/10.1126/science.aao2136>.