

# Possible Laboratory Contamination Leads to Incorrect Reporting of *Vibrio cholerae* O1 and Initiates an Outbreak Response

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***Vibrio cholerae* O1 in a river water specimen in South Africa was reported, and a public health response followed in order to prevent an outbreak. Further investigation determined this to be a pseudoalert of *V. cholerae* O1, possibly linked to laboratory contamination. Following culture of bacteria from the water specimen, the testing laboratory possibly contaminated the culture with a *V. cholerae* O1 reference strain and then mistakenly reported isolation of *V. cholerae* O1.**

Cholera is acquired by ingestion of the bacterium *Vibrio cholerae* O1 or O139 (7). Cholera has the ability to cause large, explosive outbreaks, as shown by the Haiti outbreak which started in October 2010 and recorded more than 250,000 cases and 4,000 deaths within the first 6 months (5). Therefore, a single case of *V. cholerae* O1 is notifiable in South Africa and must trigger an outbreak response, including the implementation of public health interventions. The most recent cholera outbreak in South Africa occurred over the period from November 2008 to May 2009 and recorded 12,706 cases, with 65 deaths. Since then, surveillance for *V. cholerae* O1 in South Africa over the period from June 2009 to 17 March 2011 identified only a single human case, in June 2010, which was established to have been acquired during travel in India. In the present study, we report a pseudoalert of *V. cholerae* O1 in South Africa linked to possible laboratory contamination with a laboratory reference strain.

On 18 March 2011, a water specimen was collected from a river in South Africa, as part of routine water testing done by the Department of Water Affairs. The specimen was analyzed by a local diagnostic laboratory; a strain of *V. cholerae* was isolated and identified as belonging to serogroup O1. The strain was forwarded to the Enteric Diseases Reference Unit (EDRU) of the National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service (NHLS) for further analysis. Over the period from 24 March to 1 April 2011, an additional 4 strains of *V. cholerae* were isolated from subsequent river water specimens taken in the same area as the initial *V. cholerae* O1 specimen; however, these 4 strains were identified as *V. cholerae* non-O1. These strains were also forwarded to the EDRU for further analysis. The isolation of *V. cholerae* O1 put the public health authorities on an alert and readiness for an outbreak investigation and response. Soon after, the EDRU confirmed the diagnosis (discussed below) and response activities were fully implemented. This included, first, strengthening surveillance to detect human cases and preparing health care facilities. Health care clinics and hospitals within the affected district, 4 other districts in the same province, and authorities of the neighboring province were all alerted to the findings. Health care workers were reminded of the clinical presentation and treatment of cholera and the required actions following identification of a suspected case, including telephonic notification to the local Department of Health, collec-

tion of stool specimens for laboratory investigations, and maintaining a standardized line list of all suspected cases. Second, community-level health promotion activities were initiated, and these included focus on safe-water practices and point-of-use purification where communities make use of river water for drinking, practicing good hygiene, and encouraging early health care-seeking behavior in the event of illness. Third, the Department of Water Affairs conducted further collection of water specimens from strategic sites in the catchment area to investigate the extent of contamination and a possible source. Finally, alerts were distributed to neighboring districts to sensitize them regarding cholera. Despite enhanced surveillance activities, the provincial health authorities were not notified of any suspected human cases of cholera and no clinical specimens were received by laboratories for cholera investigations.

Laboratory analysis at the EDRU proceeded as follows. The identities of bacterial isolates were investigated using standard phenotypic microbiological identification and serotyping techniques. PCR was used to determine the presence of cholera toxin (*ctxA* gene) in the bacteria and to determine the biotype of *V. cholerae* O1 by detecting the El Tor or classical variant of the *tcpA* gene (encoding the toxin-coregulated pilus), using previously described methods (8). The nucleotide sequences of *rstR*, *ctxB*, and *tcpA* genes were determined by isolation of genes from bacteria by using previously described PCR methods (3, 4, 9) followed by DNA sequencing using the BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) and an Applied Biosystems 3500 genetic analyzer. DNA sequences were collated and analyzed by using Lasergene (version 8.0) software (DNASTAR, Inc., Madison, WI), followed by examination for similarity to DNA sequences at the DNA database of the National Center for Biotechnology Information (NCBI) using the Blast algorithm

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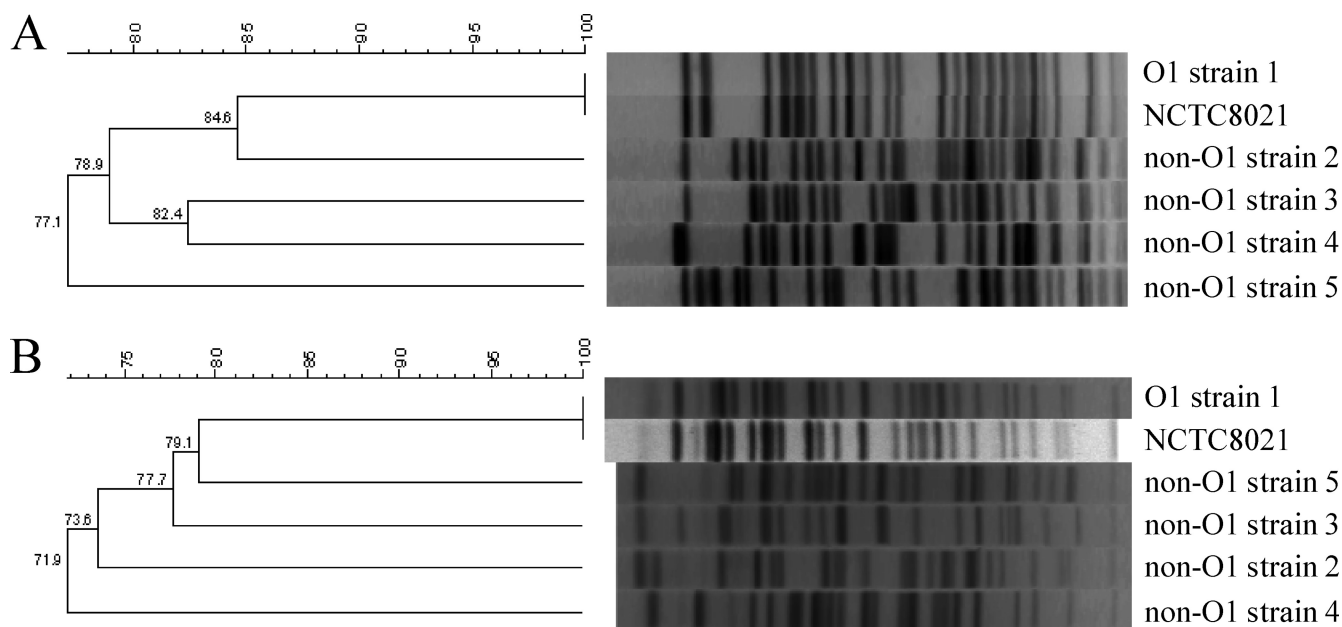


FIG 1 Dendrograms of PFGE fingerprint patterns for *V. cholerae*, following analysis with NotI digestion (A) and SfiI digestion (B).

(<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The genotypic relatedness of isolates was investigated using a PulseNet standardized pulsed-field gel electrophoresis (PFGE) analysis protocol employing separate digestion and analysis with NotI and SfiI restriction enzymes (6). PFGE patterns were analyzed by using BioNumerics (version 6.5) software (Applied Maths, Sint-Martens-Latem, Belgium) with dendrograms of the patterns created, by using the unweighted-pair group method with arithmetic averages, with analysis of banding patterns incorporating the Dice coefficient at an optimization setting of 1.5% and a position tolerance setting of 1.5%.

The first *V. cholerae* strain (strain 1) was confirmed as belonging to serogroup O1 and further determined to be serotype Ogawa and PCR positive for the cholera toxin (*ctxA* gene). Surprisingly, it was also found to be of a classical biotype, as determined by a PCR-positive result for the classical variant of the *tcpA* gene. To verify this finding of a classical biotype, the nucleotide sequence of the *rstR*, *ctxB*, and *tcpA* genes were determined for strain 1 and analyzed in the NCBI database; all DNA sequences were found to be a 100% match with DNA sequences belonging to classical *V. cholerae* O1 biotypes. The four succeeding strains of *V. cholerae* (strains 2 to 5) were confirmed as *V. cholerae* non-O1 and further determined to be PCR negative for the cholera toxin. This was unsurprising, as *V. cholerae* non-O1 strains in a patient and from water samples from the area have previously been described (12). The genetic relatedness of the five *V. cholerae* strains was then investigated by using PFGE analysis. Each strain revealed a unique PFGE pattern (Fig. 1), which suggested no close relationship between any of the strains. Interestingly, the PFGE pattern for strain 1 was unusual in that it showed no close similarity to our collection of *V. cholerae* O1 patterns (our database included only patterns of El Tor biotype strains). Our PCR and DNA sequencing results strongly suggested that strain 1 was of a classical biotype; PFGE data then supported this, as the PFGE pattern of strain 1 was uncharacteristic of typical El Tor patterns. The pattern was then

analyzed against various global databases of PFGE patterns, including the database network of PulseNet International (<http://www.pulsenetinternational.org/Pages/default.aspx>); the pattern was found to match several classical strains of *V. cholerae* O1, including a match with historical classical strains last identified in India in the 1940s and also matching more recent classical strains isolated in Mexico in the early 1990s (1). These data confirmed that strain 1 was indeed a classical biotype. Attention was now firmly focused on strain 1, and further questions arose. Additional sampling and testing of river water in the same catchment area which yielded the first isolation of *V. cholerae* O1 resulted in no further strains belonging to this O1 serogroup. Also, there was the overwhelming laboratory evidence showing that strain 1 was of a classical biotype. This was very surprising, considering that classical *V. cholerae* O1 has never been reported from Africa and that over recent years, classical strains have not been isolated worldwide, including from southern Bangladesh, the last of the niches where the classical biotype prevailed (10).

Currently, historical strains of classical *V. cholerae* O1 can be obtained from various sources, including the American Type Culture Collection (ATCC) in the United States and the National Collection of Type Cultures (NCTC), Health Protection Agency, in the United Kingdom. Reference strains from ATCC and NCTC are commonplace in South Africa testing laboratories and typically used as control strains for various laboratory tests. The testing laboratory which made the initial diagnosis of strain 1 was asked about the details of its control strains. It was found that this laboratory uses NCTC8021 (a 1940s isolate of classical *V. cholerae* O1) as a control strain. The testing laboratory was asked to forward this control strain to the EDRL for PFGE analysis. The PFGE pattern (for both NotI and SfiI digestions) was a 100% match to that of strain 1 (Fig. 1). Our investigation therefore suggests that following culture of bacteria from the river water specimen, the testing laboratory possibly contaminated the culture with a *V. cholerae* O1 control strain (NCTC8021) and then mistakenly re-

ported isolation of *V. cholerae* O1. It is not clear how this contamination may have occurred. The laboratory reports understaffing in the setting of a high workload (~9,500 tests performed during March 2011); this may well have been a contributing factor.

Pseudoalerts associated with laboratory contamination have previously been reported; a PubMed literature search of published data (limited to titles and abstracts) using the phrase “pseudo outbreak laboratory contamination” generated 24 reports dating back to 1984. Some of these reports ascribed the pseudoalerts to laboratory contamination with laboratory reference/control strains; these included a pseudo-outbreak of *Clostridium sordellii* involving six patients (2) and a pseudo-outbreak of *Mycobacterium scrofulaceum* involving three patients (11).

In conclusion, we have described a possible laboratory contamination involving a *V. cholerae* O1 reference strain, which led to a public health response in order to prevent an outbreak. This study has highlighted the fact that when a very unexpected or unusual laboratory diagnosis is made, the possibility of laboratory contamination ought to be considered. Although no true outbreak occurred, the rapid response and investigation conducted by the response teams provided a valuable exercise. This highlights the need for a fully functional laboratory, epidemiological, and managerial support structure to accurately and rapidly identify organisms of public health importance, in order to preserve limited public health resources.

**Nucleotide sequence accession numbers.** Nucleotide sequences for *rstR*, *ctxB*, and *tcpA* genes from strain 1 are not unique. Identical sequences (100% matches) are well represented and described in NCBI GenBank. As a result, sequences for strain 1 were not deposited into NCBI GenBank. Sequences for strain 1 are identical to sequences described for classical *V. cholerae* O1 strain O395, with accession numbers as follows: CP001236.1 (*rstR* and *ctxB* genes) and CP000627.1 (*tcpA* gene).

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