Possible laboratory contamination leads to incorrect reporting of *Vibrio cholerae* O1 and initiates an outbreak response

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Running head: Pseudo-alert of *V. cholerae* O1

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ABSTRACT

V. cholerae O1 was reported from a river water specimen in South Africa. A public health response followed in order to prevent an outbreak. Further investigation determined this to be a pseudo-alert 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.

TEXT

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 six months (5). Therefore, a single case of V. cholerae O1 is notifiable in South Africa (SA) and must trigger an outbreak response, including the implementation of public health interventions. The most recent cholera outbreak in SA occurred over the period November 2008 to May 2009 and recorded 12,706 cases with 65 deaths. Since then, surveillance for V. cholerae O1 in SA over the period 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 on a pseudo-alert of V. cholerae O1 in SA linked to possible laboratory contamination with a laboratory reference strain.

On 18 March 2011, a water specimen was collected from a river in SA, 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 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 later) and the response activities were fully implemented. This included, firstly, strengthening surveillance to detect human cases and preparing healthcare facilities. Healthcare clinics and hospitals within the affected district, 4 other districts in the same province and authorities of the neighbouring province, were all alerted to the findings. Healthcare 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, collection of stool specimens for laboratory investigations, and maintaining a standardised line-list of all suspected cases. Secondly, community-level health promotion activities were initiated, including: 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 healthcare seeking behaviour in the event of illness. Thirdly, 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 neighbouring districts to sensitise them regarding cholera. Despite enhanced surveillance activities, no suspected human cases of cholera were notified to the provincial health authorities and no clinical specimens were received by laboratories for cholera investigations.

Laboratory analysis at the EDRU proceeded as follows. The identity of bacterial isolates was 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 for the El Tor or classical variant of the *tcpA* gene.
gene (encoding the toxin-coregulated pilus), using previously described methods (8). The nucleotide sequence of \( \text{rstR} \), \( \text{ctxB} \) and \( \text{tcpA} \) genes were determined by isolation of genes from bacteria using previously described methods of PCR (3, 4, 9) followed by DNA sequencing using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) and an Applied Biosystems 3500 Genetic Analyzer. DNA sequences were collated and analyzed using the DNASTAR Lasergene (version 8.0) Software (DNASTAR, Inc., Madison, WI, USA), followed by examination for similarity to DNA sequences at the DNA database of the National Center for Biotechnology Information (NCBI) using the blast algorithm (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 \( \text{NotI} \) and \( \text{SfiI} \) restriction enzymes (6). PFGE patterns were analyzed using BioNumerics (version 6.5) Software (Applied Maths, Sint-Martens-Latem, Belgium) with dendrograms of the patterns created 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 \( \text{V. cholerae} \) strain (strain 1) was confirmed as belonging to serogroup O1 and further determined to be serotype Ogawa and PCR-positive for the cholera toxin (\( \text{ctxA} \) 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 \( \text{tcpA} \) gene. To verify this finding of a classical biotype, the nucleotide sequence of the \( \text{rstR} \), \( \text{ctxB} \) and \( \text{tcpA} \) genes were determined for strain 1 and analyzed at the NCBI database; all DNA sequences were found to be a 100% match with DNA sequences belonging to classical \( \text{V. cholerae} \) O1 biotypes. Regarding the other four succeeding strains of \( \text{V. cholerae} \) (strains 2 to 5), these were confirmed as \( \text{V. cholerae} \) non-O1 and further determined to be PCR-negative for the cholera toxin. This was unsurprising, as \( \text{V. cholerae} \) non-O1 has previously been described in a patient and from water samples from the area (12). The genetic relatedness of the five
V. cholerae strains were then investigated 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 only included 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. Then, 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 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 USA and the National Collection of Type Cultures (NCTC), Health Protection Agency in the UK. Reference strains from ATCC and NCTC are commonplace in SA 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 their control strains. It was found that they use NCTC8021 (a 1940s isolate of classical V. cholerae O1) as their classical strain.
V. cholerae O1) as a control strain. The testing laboratory was asked to forward this control strain to the EDRU for PFGE analysis. The PFGE pattern (for both NotI and SfiI digestions) revealed a 100% match to 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 reported 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 work-load (~9500 tests performed during March 2011); this may well have been a contributing factor.

Pseudo-alerts 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 pseudo-alerts 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, then the possibility of laboratory contamination ought to be considered. Although no true outbreak occurred, the rapid response and investigation conducted by the response teams was 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 \( \text{rstR} \), \( \text{ctxB} \) and \( \text{tcpA} \) 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 \( V. \text{cholerae} \) O1 strain O395, with accession numbers as follows: \( \text{CP001236.1} \) (\( \text{rstR} \) and \( \text{ctxB} \) genes) and \( \text{CP000627.1} \) (\( \text{tcpA} \) gene).

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Legend to Figure 1.

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