Letter to the Editor

Case of imported *Vibrio cholerae* O1 from India to South Africa

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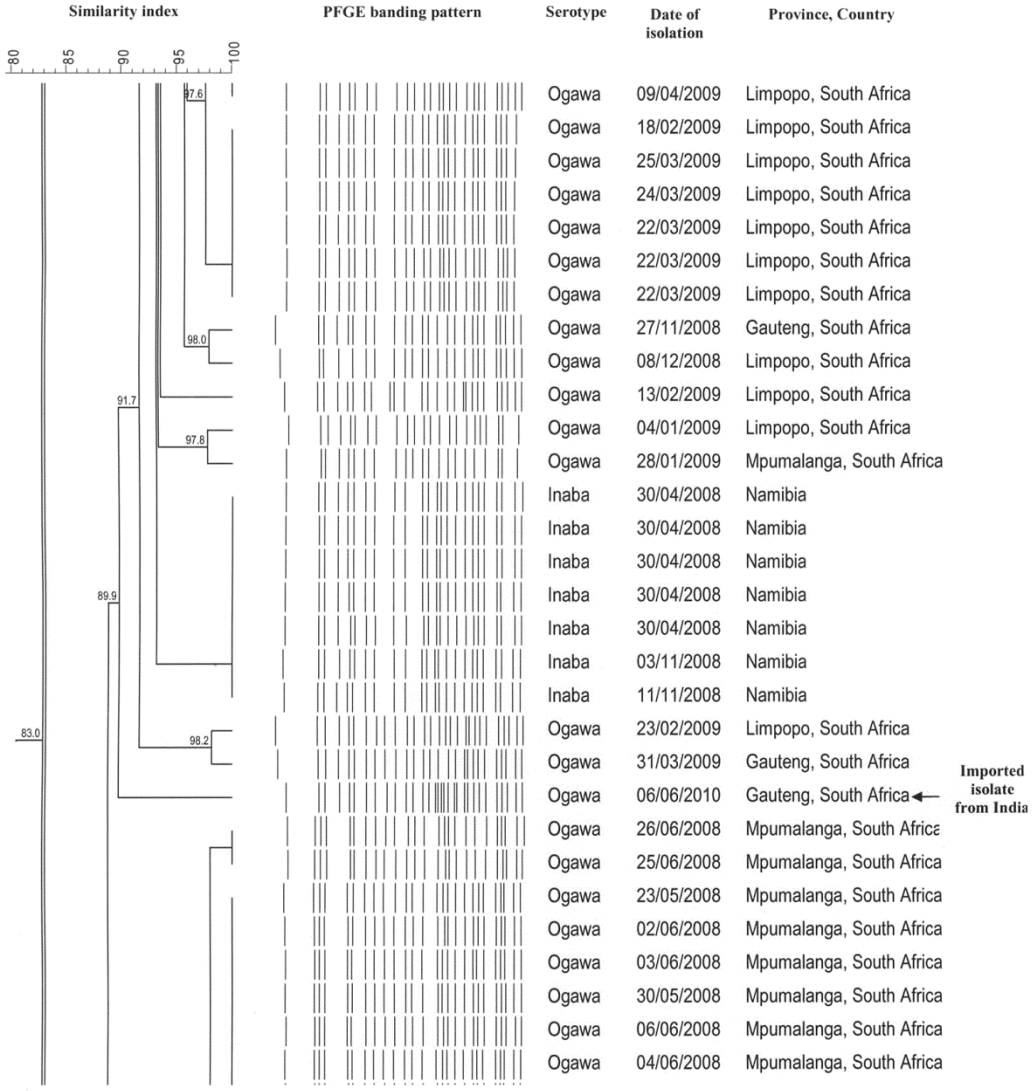
Cholera is an acute, watery diarrhoeal disease, caused by the Gram-negative bacterium *Vibrio cholerae* belonging to serogroups O1 and O139 [1]. Transmission is via the faecal-oral route through contaminated water or food [1]. Management of cholera with simple, inexpensive rehydration treatment has shown to be highly effective in reducing mortality [1,2]. Human cholera epidemics and the mortality associated with such water-borne diseases still remains a public health threat worldwide, particularly in regions with poor sanitation facilities and limited access to clean drinking water. Travellers visiting developing countries are considered at risk of contracting cholera, as these cholera endemic regions have become popular tourist destinations [1]. Here we report a confirmed case of *V. cholerae* O1, isolated from a 37-year-old female patient on her return to South Africa from India, prior to the start of the 2010 FIFA World Cup in South Africa.

A stool specimen was taken on 6 June 2010 from the patient and processed at a private laboratory in Johannesburg, South Africa. It was noted that the stool was brown in colour and of a watery consistency. Mucus, leucocytes and erythrocytes were not observed. The patient recovered, following treatment with ciprofloxacin (500 mg twice a day, for five days) and oral rehydration. *V. cholerae* O1 was isolated from the stool specimen and identified using the automated system VITEK 2 (bioMérieux, Marcy-l'Etoile, France). Isolation of *V. cholerae* O1 was a cause for concern, as South Africa was a few days away from the opening ceremony of the 2010 FIFA World Cup. The isolate was referred to the Centre for Enteric Diseases at the NICD for confirmatory testing.

The bacterial isolate was analyzed for both phenotypic and genotypic characteristics. The serogroup and serotype were confirmed by slide agglutination in polyvalent O1 and mono-specific Inaba and Ogawa antisera (Mast Diagnostics, Merseyside, United Kingdom). Minimum inhibitory concentrations with a range of antimicrobial agents were performed and interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI), 2010 guidelines [3] and breakpoints defined by Ng *et al*. [4]. PCR was used to detect the presence of both the toxin co-regulated pilus (TCP) *tcpA* [5] and the cholera toxin (CT) *ctxA* [6] respectively. Genotyping was performed by pulsed-field gel electrophoresis (PFGE) analysis using the restriction enzyme, *Not*I according to the PulseNet standardized protocol for *V. cholerae* [7]. The PFGE banding pattern was analyzed using BioNumerics (version 6.5) software (Applied Maths, Sint-Martens-Latem, Belgium) and compared to *V. cholerae* isolates collected in South Africa from 2007 to 2009 and further analyzed against a global database of *V. cholerae* PFGE patterns collected by

Figure. Section of a dendrogram of PFGE fingerprint patterns (NotI digestion) of *V. cholerae* O1 isolates collected from South Africa in recent years



the National Institute of Cholera and Enteric Diseases in India.

To better understand the genetic organization and track seventh pandemic *V. cholerae*, biotype El Tor isolates, the imported isolate together with two South African isolates, each recovered from two separate cholera outbreaks in South Africa between 2008 and 2009 [8-10], were further characterized. Extended analysis included PCR amplification and nucleotide sequencing of the complete coding regions for both the CT gene, *ctxAB* and TCP gene, *tcpA* as previously described by Talkington *et al*. [11]. DNASTAR Lasergene (version 8.0) software (DNASTAR, Inc., Madison, Wisconsin) was used to analyze the nucleotide sequences. Sequence identity for both *ctxAB* and *tcpA* genes were obtained from the DNA database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/nuccore). Two multiplex PCR assays aimed at characterizing the mobilome, which consists of genomic islands (GIs), prophages and integrative conjugative elements (ICEs) of *V. cholerae* O1 and O139, were performed as previously described by Spagnoletti *et al*. [12].

The imported bacterial isolate was confirmed as *V. cholerae* O1, serotype Ogawa and displayed resistance to streptomycin, trimethoprim-sulfamethoxazole and erythromycin. The isolate was PCR-positive for the CT (*ctxA* gene) and analysis of the *tcpA* gene showed that the cholera isolate was PCR-positive for the El

Tor variant of the TCP. The PFGE fingerprint pattern of this travel-related case was unique to the database of South African isolates (Figure) and had not been previously identified. However, when the PFGE pattern of the isolate was compared to the global database, it was shown to have a high pattern similarity (92% similarity) to *V. cholerae* O1, serotype Ogawa (El Tor hybrid) isolates associated with a cholera outbreak in Hyderabad, India, in December 2008 (data not shown). *CtxB* genotyping showed that the imported isolate expressed the classical *ctxB-7* allele [13], while both South African isolates expressed the classical *ctxB-1* allele [13]. Nucleotide sequences of the *tcpA* gene from all three isolates matched sequences from *V. cholerae* O1, biotype El Tor, strain CIRS101 (*tcpET*^{CIRS}). The mobilome of isolates was characterized by combining PCR results for two multiplex PCR reactions; the identical mobilome profile (multilocus gene profile) was determined for all three isolates, which consisted of a truncated variant of the VSP-II, an ICE*Vch*Ind5 variant and a prophage TLC [12].

In this study, PFGE analysis was able to support epidemiological data in describing how a *V. cholerae* O1 isolate was imported to South Africa from India. This isolate was not related to those from the recent outbreaks of cholera in South Africa (Figure). We have previously used the PulseNet database to track a typhoid fever outbreak across two continents [14] as it highlights the value of initiating and supporting standardized methods in developing countries. To our knowledge, this is the first report of a *ctxB-7*/*tcpET*^{CIRS} isolate recovered in South Africa. This report illustrates what many previous studies have alluded to [11,15,16]: with the advent of modern transportation and subsequent global travel, such *V. cholerae* O1 El Tor variants have the means to spread rapidly and with relative ease. Mobilome characterization data allowed us to describe and track the source of all three isolates. The sole mobilome profile obtained in this study matched the same profile (profile B) as the reference isolate, *V. cholerae* O1 strain CIRS101 (*ctxB-1*/*tcpET*^{CIRS}), isolated in Dhaka, Bangladesh in 2002 [11,12]. This strain is currently considered to be the most successful variant, as isolates belonging to this type have almost replaced the prototype El Tor in Asia and many parts of Africa, particularly in East Africa [17].Global networks, such as PulseNet, and standardized strategies for characterizing pathogenic isolates are invaluable to the understanding of how enteric pathogens travel globally as well as for identifying and managing potential outbreaks of diseases such as cholera.

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