Short communication

Vibrio cholerae O1 Ogawa El Tor strains with the ctxB7 allele driving cholera outbreaks in south-western India in 2012


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Abstract

Cholera has been a recurrent epidemic disease in human populations for the past 200 years. We present herein a comparative characterization of clinical Vibrio cholerae strains isolated from two consecutive cholera outbreaks in 2012 and associated environmental strains from western India. The clinical and toxigenic environmental isolates were identified as hybrid V. cholerae O1, serotype Ogawa, biotype El Tor carrying the variant ctxB7 allele. Partial sequences of SXT integrase from the isolates revealed 100% identity to ICEVchInd5 (Sevagram, India, 1994) and VC1786ICE (Haiti, 2013). The full clonal relationship of the strains established by RAPD, Box PCR, ERIC PCR and MLST (pyrH, recA, and rpoA) analyses, and the short time between the two outbreaks, strongly supported that both outbreaks were due to a single strain. The study corroborated that faecal contamination of the potable water supply was the main reason for the first outbreak, which further spread to other areas and resulted in the second outbreak. The study concluded that the circulating El Tor variant strains of epidemic potential in the region can be a serious concern in the future.

1. Introduction

Cholera, an ancient waterborne disease, continues to be a devastating disease globally with a greater prevalence in developing nations. Vibrio cholerae is primarily an inhabitant of the aquatic environment, so water plays an important role in the transmission and epidemiology of cholera. The recent outbreak reports from India, Angola, Sudan, Zimbabwe, Nigeria and Haiti have revealed that the mortality resulting from the disease has far exceeded the World Health Organization (WHO) international target of 1% or less (Enserink, 2010). Several outbreaks and sporadic cases of cholera have been noted from time to time in India. V. cholerae O1 has two biotypes: classical (more toxigenic) and El Tor (more resistant to environmental factors). It has genetic plasticity and continues to evolve in virulence and drug resistance, thus ensuring its survival and persistence. This leads to the emergence of new hybrid or variant strains (e.g. El Tor strains have captured the toxin of the classical type), hampering disease control policies. El Tor variant strains have better colonization efficiency and are able to produce a significantly higher amount of cholera toxin (CT) in vivo or in virulence-induced conditions (Ghosh-Banerjee et al., 2010). Moreover, an El Tor variant strain emerged with a novel CT (ctxB7 allele), first reported in Orissa; later it rose in fame as Haitian ctxB (HCT) after causing a devastating epidemic in Haiti (Kumar et al., 2009; Naha et al., 2012; Kutar et al., 2013). Earlier, we noted a cholera outbreak in Yavatmal district of Maharashtra associated with HCT (Kumar et al., 2013). In the present study, we aimed to link the outbreak to equipment damage in the drinking water supply and contamination of sewer water, associated with back-to-back cholera outbreaks in the same district of Maharashtra, south-western India. We determined the molecular epidemiological traits and phylogenetic relationship of V. cholerae isolates from the two consecutive cholera outbreaks with environmental strains.

2. Materials and methods

2.1. Isolation and characterization of bacterial strains

A total of 20 clinical V. cholerae isolates were recovered from randomly selected patients (unrelated individuals) during cholera outbreaks in the month of May, 2012 from Kalamb (10 isolates) and Yavatmal (10 stool samples), Maharashtra, India (see the
KML file attached as a Supplement). The patients were admitted to the Shri VasantaNaik Government Medical College, Yavatmal. The stool samples collected using sterile swabs were processed as described earlier (Pourshafie et al., 2007). Meanwhile, the drinking water supply to the town, which was connected to a water purification plant on the banks of Wardha River, was monitored. Water samples (sewage and potable) were collected from the outbreak-affected areas and neighbouring houses. A 1-L water sample was filtered through a 0.45-μm membrane using a vacuum pump, the membrane was enriched in alkaline peptone water for 6 h followed by plating on TCBS agar. The environmental and clinical isolates were screened for the presence of the CT gene by colony polymerase chain reaction (PCR). All the toxigenic (CT-positive) isolates (five environmental and 20 clinical) were further subjected to serotyping and biotyping (Jain et al., 2011).

2.2. Screening of virulence-associated gene traits

Genomic DNA was isolated from all the selected toxigenic isolates using a genomic DNA purification kit (MBI Fermentas, Vilnius, Lithuania) and screened for the presence of genetic traits associated with virulence and biotype: ompW, ctxAB, zot, rfbO1, tcp, ace, hlyA, ompU, rtx, toxR, tcpX and rtxC allele using PCR as described earlier (Kumar et al., 2009; Jain et al., 2011). Other virulence genes – vibrio pathogenicity island (VPI), aldA and tagA – were detected by single PCR assay. The ctxB gene was amplified from the clinical and environmental isolates and subjected to DNA sequencing and ctxB typing (Jain et al., 2011). Organization and chromosomal localization of the CTX prophage was determined by PCR using specific primers reported earlier (Nguyen et al., 2009; Jain et al., 2011). Details of all the primers used in this study are given in Supplementary Table S1.

2.3. Analyses of SXT constin

The presence of SXT constin was determined by PCR amplification using the primers SXT-F and SXT-R directed to the SXT element and int1-F and int1-B primers targeted for SXT integrase. The amplicon of SXT-F/SXT-R was purified and sequenced. The strains were screened for floR presence on SXT constin and the antimicrobial susceptibility test for chloramphenicol (Sjolund-Karlsson et al., 2011).

2.4. Genetic relatedness of the strains by DNA fingerprinting and multilocus sequence typing

The phylogenetic relationship of the clinical and environmental isolates was analysed by enterobacterial repetitive intergenic consensus (ERIC) PCR, BOX-PCR and random amplified polymorphic DNA (RAPD) PCR (Kumar et al., 2009). The strains were further subjected to multilocus sequence typing based on pyrH, recA and rpoA genes using MEGA5 according to a previously described method (Thompson et al., 2011).

3. Results and discussion

3.1. Characterization of V. cholerae strains

All the clinical and toxigenic environmental V. cholerae isolates were identified as V. cholerae O1 Ogawa. Biochemical and PCR assays confirmed the El Tor biotype. PCR analyses revealed the presence of other virulence-associated traits such as ctxAB, tcpA, zot, ace, toxR, rfbO1 and VPI genes (Table 1). The presence of toxigenic strains in environmental water is an indication of an
impending threat of cholera outbreak due to contamination of drinking water (Singh et al., 2001).

3.2. ctxB genotyping and CTX prophage array

DNA sequencing identified the ctxB7 allele carrying Asn instead of His at the 20th position in a signal peptide. The PCR amplification of the rstC gene confirmed the presence of RS1-CTX prophage array in all the isolates. It was present only on the large chromosome in the strains as indicated by PCR amplification with ch1F/rstAR and ctxBF/ch1R primers. All the isolates belonged to Group II and contained the RS1 and CTX prophage (Fig. S1) harbouring the El Tor type of rstR and the classical type of ctxB on the large chromosome, a genetic map similar to the Vietnamese strain (Nguyen et al., 2009). Based on RS1-CTX prophage, atypical El Tor strains have been classified into two groups: Group 1 (tandem repeat of the classical CTX prophage on the small chromosome) and Group 2 (the RS1-CTX prophage with the El Tor type rstR and classical ctxB on the large chromosome) (Lee et al., 2009).

The altered biotype in V. cholerae O1 El Tor (classical CT) strains has been reported from different geographical regions such as Bangladesh, Mozambique, Vietnam, Hong Kong, Japan, Zambia, India, Africa, Nigeria and Haiti (Safa et al., 2010; Marin et al., 2013). The hybrid property enhances the infection potential of the bacterium and such strains are associated with more fluid loss and a higher case fatality rate. In India, these strains were reported to cause repeated outbreaks in different parts of India including Orissa, Chennai, Hyderabad, Solapur and Assam (Goel and Jiang, 2010; Jain et al., 2011; Borkakoty et al., 2012). The HCT was first reported from a large cholera outbreak in Orissa and later it was found associated with epidemics in Africa and Haiti. It was also noted in strains collected from sporadic cholera cases in Kolkata and Yavatmal; in the latter it was found to be persistent in the region after causing an outbreak in Kalamb (Kumar et al., 2013; Kumar et al., 2013).

3.3. Analysis of SXT constin

Partial sequences of the SXT integrase of the isolates were submitted to GenBank with Accession No. KF290492-94. The sequences were 100% identical to SXT integrase of strains from ICE-VchInd5 from Sevagram (GQ463142) and Kolkata, India, and VC1786ICE from the Haiti outbreak strains (JN648379). ICEVchInd5 is a prevalent SXT element in Indian V. cholerae O1 El Tor strains and was first described in the El Tor strain collected from Sevagram (Wardha) in 1994, and a similar SXT element (ICEvchHA1) was found in Haitian strains (Ceccarelli et al., 2011). Wardha and Yavatmal are two adjacent districts of Maharashtra state, showing that V. cholerae with an SXT element continues to prevail in the state and is disseminated to distinct geographical areas. The strains were found positive for floR present on SXT constin, conferring the resistance against chloramphenicol. The floR was not associated with resistance in the isolates because the minimum inhibitory concentration (MIC) for chloramphenicol was 4–8 μg/mL. The genotype (floR) with a similar phenotype has also been observed in the isolates that caused the worst epidemic in recent years in Haiti (Sjolund-Karlsson et al., 2011).

3.4. Genetic relatedness of the strains

Three DNA fingerprinting methods – random amplified polymorphic DNA (RAPD), Box and ERIC PCR – generated identical DNA banding patterns among the clinical and environmental strains, revealing their clonal relationships. The multilocus sequence typing (MLST) results also revealed single clusters for clinical and environmental strains along with the pandemic V. cholerae O1 El Tor strain (N16961) and strains from Haiti and Ghana. Identical DNA sequences were observed in all the strains for each of the three loci (pyrH, recA, rpoA) (Fig. 1). Only three representative strain’s concatenated nucleotide sequences, one from each group – Kalamb (VCK1201), Yavatmal (VCY1257) and environmental (VEK1235) – were included in phylogenetic tree construction. The clonality of the strains points towards a single source of origin, indicating that a single clone of V. cholerae was responsible for both outbreaks. Earlier reports established the clonal relationship of environmental strains with clinical strains, demonstrating that the environmental reservoir of V. cholerae is the cause of cholera outbreaks (Singh et al., 2001). Our previous investigations from Orissa, Hyderabad, Chennai and Solapur also revealed the involvement of a single clone in the respective outbreaks (Goel and Jiang, 2010; Jain et al., 2011; Kumar et al., 2009). The drinking water supplied to Kalamb passed through a sewer drainage system near the most severely affected houses. Leakage in the supply pipeline at the cross-point of sewer drainage near a house undergoing renovation works was observed. It might have allowed suction of sewage water into the drinking water during the supply off-period due to creation of negative pressure. The presence of clonal toxigenic strains in drinking water and associated sewage water supports this hypothesis. The faecal contamination of the potable water supply might be the major reason for the first outbreak. The strains remained persistent in the area after the first outbreak. Subsequently, the strains would have spread to other areas in the district associated with the second outbreak.

4. Conclusions

In the present study, all the clinical and toxigenic environmental isolates of V. cholerae O1 El Tor share their biotype with the CT
variant, showing their epidemiological and environmental success. The clonality of environmental and clinical strains based on DNA fingerprinting and MLST analyses revealed a common origin and highlighted the role of drinking water contamination in an eruption of a cholera outbreak. The 100% identity in a partial sequence of SXT integrase with ICEVchInd5 (Sevagram, India) and VC1786ICE (Haiti) confirmed that the strains still prevail in the state and have been disseminated to other geographical regions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2014.03.020. These data include Google maps of the most important areas described in this article.

References


