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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 (*ctx*B7 allele), first reported in Orissa; later it rose in fame as Haitian *ctx*B (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

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KML file attached as a Supplement). The patients were admitted to the Shri Vasantrao Naik 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*, *rtxC* and $rstR^{ElTor}$ 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 sets of 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 *flo*R 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

Table 1
Biotype and virulence associated chracteristics.

Origin/year of isolation	Location of isolation	Strain type	No. of strains	Biotype specific characteristics						Virulence associated genotype	
				VP test	PB	Haemolysis	*ctxB*	*rstR*	*rtxC*	mPCR1	mPCR2
India, 1948	569B	O1, Classical	1	–	S	–	C	C	–	*OmpW*$^+$, *ctxAB*$^+$, *rfbO1*$^+$, *tcp*$^+$, *zot*$^+$	*rtxC*$^-$, *ace*$^+$, *hlyA*$^-$, *OmpU*$^+$, *toxR*$^+$
Bangladesh, 1971	N16961	O1, El Tor	1	+	R	+	E	E	+	*OmpW*$^+$, *ctxAB*$^+$, *rfbO1*$^+$, *tcp*$^+$, *zot*$^+$	*rtxC*$^+$, *ace*$^+$, *hlyA*$^+$, *OmpU*$^+$, *toxR*$^+$
Clinical/2012	Kalamb, Maharashtra	O1, El Tor	10	+/–	R	+	C	E	+	*OmpW*$^+$, *ctxAB*$^+$, *rfbO1*$^+$, *tcp*$^+$, *zot*$^+$	*rtxC*$^+$, *ace*$^+$, *hlyA*$^+$, *OmpU*$^+$, *toxR*$^+$
Clinical/2012	Yavatmal, Maharashtra	O1, El Tor	10	+/–	R	+	C	E	+	*OmpW*$^+$, *ctxAB*$^+$, *rfbO1*$^+$, *tcp*$^+$, *zot*$^+$	*rtxC*$^+$, *ace*$^+$, *hlyA*$^+$, *OmpU*$^+$, *toxR*$^+$
Environmental/2012	Kalamb, Maharashtra	O1, El Tor	5	+/–	R	+	C	E	+	*OmpW*$^+$, *ctxAB*$^+$, *rfbO1*$^+$, *tcp*$^+$, *zot*$^+$	*rtxC*$^+$, *ace*$^+$, *hlyA*$^+$, *OmpU*$^+$, *toxR*$^+$

–: negative, +: positive, +/–: weak positive, R: resistant, S: susceptible, C: classical, E: El Tor, VP: Voges Proskauer, PB: Polymyxin B; mPCR1 (multiplex PCR1), mPCR2 (multiplex PCR2) (Kumar et al., 2009); Clinical strain from Kalamb have been earlier characterized by Kumar et al. (2013).