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A new integrative conjugative element detected in Haitian isolates of *Vibrio cholerae* non-O1/non-O139

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Abstract

The presence of SXT/R391-related integrating conjugative elements (ICEs) in *V. cholerae* O1 and non-O1/non-O139 isolated from clinical and environmental samples in Haiti in 2010 was studied. The main finding of this work was the identification of the novel ICE*Vch*Hai2 among closely related *V. cholerae* non-O1/non-O139 clinical strains. The mosaic structure of this element confirms the role of ICEs as efficient recombination systems whereby new genetic material can be acquired and exchanged, according *V. cholerae* strains new accessory functions.

Keywords

Vibrio cholera; Non-O1/non-O139; Haiti; Integrative conjugative elements

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Vibrio cholerae is an autochthonous inhabitant of riverine and estuarine environments. It is a facultative pathogen of humans, being the causative agent of cholera, a disease endemic in many developing countries (Colwell, 1996). Since October 2010, when the first case of cholera was diagnosed in Haiti, cholera remains a major health threat, with more than 600,000 cases to date (Ministère de la Santé Publique et de la Population, 2013).

Integrative conjugative elements (ICEs) of the SXT/R391 family are recognized for their role in bacterial genome plasticity and as vectors of antibiotic resistance and alternative metabolic pathways (Seth-Smith and Croucher, 2009). They share a conserved genetic scaffold of 52 genes encoding their own conjugation, integration, excision, and regulatory machinery (Wozniak et al., 2009). The backbone is scattered with accessory sequences located in conserved regions (hotspots) and exhibiting significant variability in genetic content, including resistance to antibiotics and heavy metals, toxin/antitoxin systems, restriction/modification systems, motility and biofilm formation (Bordeleau et al., 2010; Wozniak et al., 2009).

SXT/R391 ICEs are ubiquitous in clinical *V. cholerae* O1 and O139, as well as in other vibrios (Wozniak et al., 2009), and their acquisition has been recognized as a distinctive trait in the ongoing evolution of pandemic *V. cholerae* strains (Mutreja et al., 2011). Our knowledge of the distribution of SXT/R391 ICEs in environmental isolates is still very limited. To date, SXTR391 ICEs were detected in few *Gammaproteobacteria* and *Vibrio* spp. from aquaculture and marine environments (Badhai et al., 2013; Osorio et al., 2008; Rodriguez-Blanco et al., 2012) and in *V. cholerae* non-O1/non-O139 environmental isolates from Mexico and Mozambique (Taviani et al., 2008; Wozniak et al., 2009).

In this work, we report ICE analysis of *V. cholerae* O1 and non-O1/non-O139 isolated from clinical and environmental samples collected in the early phase of the cholera outbreak in Haiti in 2010 (Hasan et al., 2012). We analyzed 57 sequenced strains that had been isolated in 11 arrondissements of Haiti: 30 clinical *V. cholerae* O1 and 27 non-O1/non-O139 strains, the latter of clinical and environmental origin. ICE sequence and genetic organization were compared with other SXT/R391 ICEs in GenBank using tools previously described (Taviani et al., 2012).

Based on occurrence and genetic structure of their SXT/R391 ICEs, the Haitian strains can be divided into three clusters. All *V. cholerae* O1 clinical strains contain an ICE that is >99% similar to ICE*Vch*Ind5 (a.k.a. ICE*Vch*Hai1), as previously reported for *V. cholerae* O1 strains from the same epidemic (Ceccarelli et al., 2011c; Sjolund-Karlsson et al., 2011). This is not surprising considering that ICE*Vch*Ind5 and siblings are prevalent in epidemic *V. cholerae* O1 altered El Tor variants worldwide (Ceccarelli et al., 2011b; Ceccarelli et al., 2011a).

The non-O1/non-O139 strains, in comparison, showed two profiles. Twelve strains, both environmental and clinical, were devoid of SXT/R391 ICEs. Those clinical strains were isolated from the arrondissements of Croix-des-Bouquets, Delmas and Port-au-Prince in the Ouest department, and formed a closely related cluster (Hasan et al., 2012), whereas

environmental isolates were from gray water, latrines and hospital waste in Cange, in the Central Plateau of Haiti.

The main finding of this work is identification of the novel ICE*Vch*Hai2 in 15 non-O1/non-O139 *V. cholerae* clinical strains isolated both in the Artibonite and Ouest departments. The 15 ICEs share almost 100% similarity at the nucleotide level and are separated only by one single nucleotide polymorphism (SNP) among the whole sequence. As noted for all SXT-related ICEs, the genetic structure of ICE*Vch*Hai2 (GenBank Accession No. AJRO01000008, nt. 45958-128752, 82.795 bp) is a genetic mosaic shaped by inter-ICE recombination (Garriss et al., 2009; Wozniak et al., 2009). We identified gene sequences from other ICEs as well as unique genetic clusters (Figure 1). Like all environmental ICEs described to date in *V. cholerae* (Taviani et al., 2008; Wozniak et al., 2009), ICE*Vch*Hai2 lacks the antibiotic resistance cluster typically inserted in variable region 3 (VR3). Hotspot 1 and variable region 1 (VR1) have the same molecular rearrangement as ICE*Vch*Mex1. Among other genes, hotspot 3 of ICE*Vch*Mex1 encodes *dgcK* and *dgcL* involved in the c-di-GMP signaling pathways (Bordeleau et al., 2010). ICE*Vch*Hai2 contains only *dgcK* with an inverted orientation compared to ICE*Vch*Mex1, and the rest of hotspot 3 is deleted. Also, hotspot 2 appears to be the result of a deletion event. It contains only two genes (*orf45*, *orf46*) found in both R391 and ICE*Vch*Mex1, but the rest of the hotspot is missing. Hotspot 4, located between genes *traN* and *s063*, is characterized by a unique 9.4 kb region that includes nine ORFs. Bioinformatics analysis of its genetic content revealed three hypothetical proteins and two transposase genes. The remaining five genes are associated with known functions involved in DNA-mediated transposition, transformation or recombination, as well as deoxyribonuclease activity and nucleic acid binding (Table 1). Finally, hotspot 5 revealed 96% homology with the same region in ICE*Vch*Ind5, a 14.8-kb hypothetical operon of unknown function (Ceccarelli et al., 2011b).

The results of our analysis show that Haitian *V. cholerae* strains contain two SXT/R391 ICEs displaying different genetic organization: ICE*Vch*Ind5 and ICE*Vch*Hai2. ICE*Vch*Hai2 is described here for the first time, appears to circulate only among closely related *V. cholerae* non-O1/non-O139 clinical strains (Hasan et al., 2012) and shares several hotspots with ICE*Vch*Mex1, isolated in non-O1/non-O139 *V. cholerae* from sewage in Mexico (Wozniak et al., 2009). Yet a general phenomenon of recombination appears to have occurred in ICE*Vch*Hai2 compared with ICE*Vch*Mex1. The Haitian ICE shows deletions in homologous hotspot regions and bears new genes involved in recombination and nucleic acid binding and processing. These findings indicate that the ICE*Vch*Hai2 hotspots have been deleted or rearranged without compromising the integrity of core genes required for ICE mobility and ability to acquire DNA. Indeed, ICE*Vch*Hai2 may have a function yet to be determined, but confirms the role of SXT/R391 ICEs as efficient recombination systems whereby new genetic material can be acquired and exchanged, according new ICE variants with different accessory functions.

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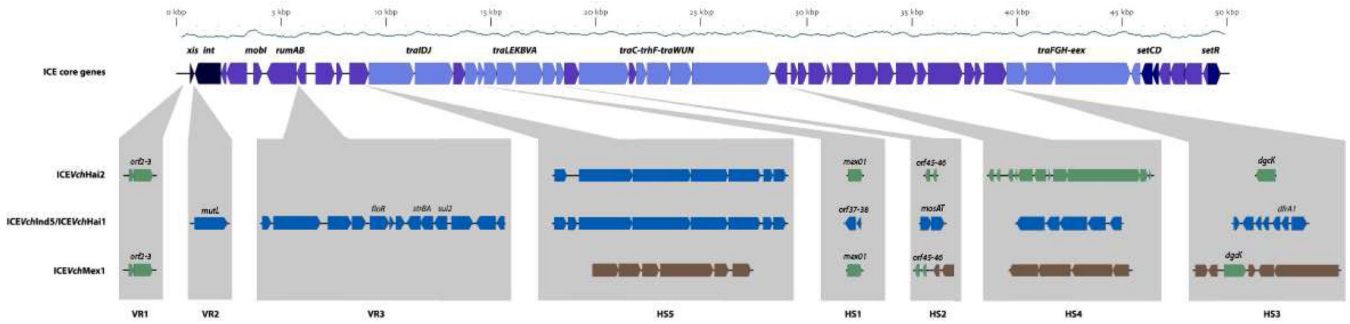

Fig. 1. Structural comparison between ICE*Vch*Ind5 (ICE*Vch*Hai1), ICE*Vch*Hai2 and ICE*Vch*Mex1

The upper map represents the set of core genes common to all SXT/R391 ICEs. Highlighted are genes involved in site-specific excision and integration (*xis*, *int*, *mobI*), error prone DNA repair (*rumAB*), entry exclusion (*eex*), ICE regulation (*setCDR*) and conjugative transfer (*tra* genes). The bottom map represents the specific regions of ICE*Vch*Ind5 (ICE*Vch*Hai1), ICE*Vch*Hai2, and ICE*Vch*Mex1 inserted the variable regions and hotspots. For a detailed description of hotspot 4 in ICE*Vch*Hai2 see Table 1.

Table 1

ORF content of hotspot 4 in ICE*Vch*Hai2

Annotated genes refer to ICE*Vch*Hai2 in *V. cholerae* HC-1A2 (GenBank Accession No. AJRO01000008.1). Blast2Go was used to perform functional annotation with Gene Ontology (GO) and InterProScan.

Gene	Putative role	Gene Length (nt)	GO terms
VCHC1A2_1617	Transposase	327	Transposition, DNA-mediated
VCHC1A2_1618	Polyribonucleotide nucleotidyltransferase	141	Transposition, DNA-mediated
VCHC1A2_1619	*trp* Repressor family protein	951	Nucleic acid binding
VCHC1A2_1620	Transposase dde domain protein	759	Transposition, DNA-mediated
VCHC1A2_1621	Hypothetical protein VCHC1A2_1621	126	-
VCHC1A2_1622	*smf* family protein	930	DNA-mediated transformation
VCHC1A2_1623	ATP-dependent DNA helicase	4677	DNA recombination
VCHC1A2_1624	Hypothetical protein VCHC59A1_0788	489	-
VCHC1A2_1625	Hypothetical protein VCHC1A2_1625	117	-
VCHC1A2_1626	Endonuclease-1	678	Deoxyribonuclease I activity