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Correspondence

Haitian variant *tcpA* in *Vibrio cholerae* O1 El Tor strains in National Capital Region (India)

Sir,

Vibrio cholerae O1 is the causative agent of cholera, which has two biotypes, namely, classical and El Tor, based on various phenotypic and genotypic characters[1]. Cholera toxin (*ctx*) and toxin co-regulated pilus (*tcp*) are essential virulence genes. The expression of CT and TCP is regulated by ToxR, a co-regulatory protein[2]. TCP is a type IV pilus which is essential for colonization in the small intestine[3]. Whole genome sequence of *V. cholerae* strains isolated from Bangladesh (CIRS101) and Haitian outbreak has shown a single nucleotide polymorphism (SNP) at a nucleotide position 266 (amino acid 89) of the *tcpA* gene, particularly associated with the Haitian variant[4-6]. We undertook this study to investigate the presence of mutation in *tcpA* allele in *V. cholerae* El Tor strains obtained from National Capital Region (NCR) of India.

A total of 71 *V. cholerae* strains could be revived from the collection maintained in the Laboratory Department of Maharishi Valmiki Infectious Diseases Hospital (MVIDH), Delhi, India. These strains were collected from NCR (Delhi, Haryana and Uttar Pradesh) during 2008-2012 and were characterized biochemically and serotyped as *V. cholerae* O1 Ogawa using standard procedure[7]. Environmental sampling and processing of samples were done according to Mishra *et al*[8]. A total of 204 samples were collected which included 171 drinking water samples from the houses of patients admitted to MVIDH, 13 samples from Najafgarh drain, seven samples from different lakes and three samples from the Yamuna river. Only two drinking water samples and one sewage samples were positive for *V. cholerae* O1. Detection of the mutation in *tcpA* gene encoding TCP was done by a polymerase chain reaction (PCR) assay[9]. This PCR assay discriminates the *V. cholerae* strains harbouring Haitian, classical and El Tor alleles of *tcpA*, and this may be used to understand the presence of the new variant in different areas of cholera endemicity.

In this study, three different primers used included one common reverse primer for both El Tor and Haitian type *tcpA* alleles [*tcpA* El-Rev (5'-CCGACTGTAATTGCGAATGC-3')]. Two forward primers [*tcpA*-F'1 (5'-CCAGCTACCGCAAACGCAGA-3') and *tcpA*-F'2 (5'-CCAGCTACCGCAAACGCAGG-3')] specific for El Tor and Haitian type *tcpA* alleles were used, respectively. The PCR assay conditions and PCR cycles were as described previously[9]. N16961 was used as a control strain for El Tor, and EL-1786 for Haitian, to check the mutation in *tcpA* gene. These control strains were obtained from National Institute of Cholera and Enteric Diseases (NICED), Kolkata, India.

PCR assay confirmed all the 71 strains carrying *tcpA* of Haitian type which yielded a 167bp fragment with Haitian-specific primer pair but not with El Tor-specific primer. Only *V. cholerae* O1 Inaba El Tor biotype (control strain N16961) was amplified with El Tor-specific primers which yielded a 167bp fragment but not with Haitian *tcpA*-specific primer. Previous studies reported a single nucleotide change at 266 position which resulted in asparagine to serine substitution[4,9]. This mutation (Asn→Ser) at the 89th amino acid of whole *tcpA* was the result of transition which took place in isolates of Bangladesh in 2002[10] and isolates of Kolkata, India, in 2003[9]. Haitian *tcpA* allele (*tcpET*^{CIRS}) has been found among the isolates of Afghanistan, Cameroon, India, Nepal, Nigeria, Pakistan, South Africa and Sri Lanka[5]. In this study, the combination of Haitian *ctxB* (*ctxB7*), classical *ctxB* (*ctxB1*) and *tcpA* of Haitian allele is reported for the first time since 2008 in north India including Delhi, Haryana and Uttar Pradesh in both 69 clinical and two environmental isolates (sewage & drinking water) (Table). The presence of *tcpA* Haitian

Table. The list of clinical (n=69) and environmental (n=2) *Vibrio cholerae* strains based on polymerase chain reaction study isolated from diarrhoeal patients in north India

Strain ID	Year of isolation	Source	Place/State	*tcpA*	*ctxB*
DL8369	2008	Clinical	Delhi	Haitian	Negative
DL9040	2008	Clinical	Delhi	Haitian	Classical
DL12566	2009	Clinical	Delhi	Haitian	Haitian
DL20045	2010	Clinical	Delhi	Haitian	Classical
DL26903	2011	Clinical	Delhi	Haitian	Haitian
DL30167	2012	Clinical	Delhi	Haitian	Haitian
DL30209	2012	Clinical	Delhi	Haitian	Haitian
DL30235	2012	Clinical	Delhi	Haitian	Haitian
DL30259	2012	Clinical	Delhi	Haitian	Haitian
DL30321	2012	Clinical	Delhi	Haitian	Haitian
DL30453	2012	Clinical	Delhi	Haitian	Haitian
DL30492	2012	Clinical	Delhi	Haitian	Haitian
DL30534	2012	Clinical	Delhi	Haitian	Haitian
DL30743	2012	Clinical	Delhi	Haitian	Haitian
DL30744	2012	Clinical	Delhi	Haitian	Haitian
DL30755	2012	Clinical	Delhi	Haitian	Haitian
DL30793	2012	Clinical	Delhi	Haitian	Haitian
DL30800	2012	Clinical	Delhi	Haitian	Haitian
DL30894	2012	Clinical	Delhi	Haitian	Haitian
DL30908	2012	Clinical	Delhi	Haitian	Haitian
DL30914	2012	Clinical	Delhi	Haitian	Haitian
DL30946	2012	Clinical	Delhi	Haitian	Haitian
DL30970	2012	Clinical	Delhi	Haitian	Haitian
DL31035	2012	Clinical	Delhi	Haitian	Haitian
DL31040	2012	Clinical	Delhi	Haitian	Haitian
DL31073	2012	Clinical	Delhi	Haitian	Haitian
DL31221	2012	Clinical	Delhi	Haitian	Haitian
DL31235	2012	Clinical	Delhi	Haitian	Haitian
DL31248	2012	Clinical	Delhi	Haitian	Haitian
DL31286	2012	Clinical	Delhi	Haitian	Haitian
DL31941	2012	Clinical	Delhi	Haitian	Haitian
DL31998	2012	Clinical	Delhi	Haitian	Haitian
DL32165	2012	Clinical	Delhi	Haitian	Haitian
DL32179	2012	Clinical	Delhi	Haitian	Classical
DL32197	2012	Clinical	Delhi	Haitian	Classical
DL32521	2012	Clinical	Delhi	Haitian	Haitian
DL32524	2012	Clinical	Delhi	Haitian	Classical
DL32639	2012	Clinical	Delhi	Haitian	Haitian
DL32724	2012	Clinical	Delhi	Haitian	Haitian
DL32747	2012	Clinical	Delhi	Haitian	Haitian
DL32803	2012	Clinical	Delhi	Haitian	Haitian

Contd...

Strain ID	Year of isolation	Source	Place/State	*tcpA*	*ctxB*
DL32812	2012	Clinical	Delhi	Haitian	Haitian
W-984	2012	Drinking water	Delhi	Haitian	Haitian
ND-II	2012	Sewage water	Delhi	Haitian	Haitian
HR31698	2012	Clinical	Haryana	Haitian	Classical
HR31853	2012	Clinical	Haryana	Haitian	Classical
HR31860	2012	Clinical	Haryana	Haitian	Classical
HR31948	2012	Clinical	Haryana	Haitian	Classical
HR32285	2012	Clinical	Haryana	Haitian	Haitian
HR32286	2012	Clinical	Haryana	Haitian	Haitian
HR32289	2012	Clinical	Haryana	Haitian	Haitian
HR32355	2012	Clinical	Haryana	Haitian	Haitian
HR32375	2012	Clinical	Haryana	Haitian	Classical
HR32394	2012	Clinical	Haryana	Haitian	Classical
HR32626	2012	Clinical	Haryana	Haitian	Haitian
HR33102	2012	Clinical	Haryana	Haitian	Haitian
UP31298	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP31299	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP31521	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP32016	2012	Clinical	Uttar Pradesh	Haitian	Classical
UP32062	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP32198	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP32200	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP32208	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP32259	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP32268	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP32318	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP32333	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP32441	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP32562	2012	Clinical	Uttar Pradesh	Haitian	Haitian
UP32665	2012	Clinical	Uttar Pradesh	Haitian	Haitian

allele was not restricted to strains having *ctxB7* allele. It was equally present among both *ctxB1* and *ctxB7* isolates. Such molecular intricacies are important to understand the contemporary developments taking place in *ctxB* and *tcpA* genes globally. Keeping in view of the Haiti experiences and findings in our country, there is a need for the redressal of control strategies being adopted in the surveillance of cholera disease in this endemic region.

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