Cholera, caused by *Vibrio cholerae*, is a major public-health concern in most developing countries of Asia and Africa. Of more than 200 serogroups identified, based on the variation of ‘O’ antigenic LPS, *V. cholerae* serogroups O1 and O139 are known to be associated with the epidemics and pandemics of cholera. *V. cholerae* O1 has two biotypes, classical (CL) and El Tor (ET), differing in major phenotypic and genetic traits (Safa *et al.*, 2010), including the cholera toxin (CT) prophage (CTXφ) encoding the potent toxin responsible for the deadly disease cholera. The CT has two subunits, the enzymic subunit ‘A’ (*ctxA*) and the receptor binding subunit ‘B’ (*ctxB*). The *ctxB* genotyping scheme was adopted based on the amino acid substitution at position 39, 46 and 68, which showed *ctxB*1 was conserved in the CL biotype and the US Gulf Coast ET, *ctxB*2 was found in Australian ET, and *ctxB*3 in the seventh pandemic ET and Latin American ET strains (Olsvik *et al.*, 1993). Over the last two decades, 12 different *ctxB* genotypes, and a subtype with an extra 11 amino acid repeat designated *ctxB*3b, have been reported from different serogroups of *V. cholerae*. The genotypes *ctxB*1, *ctxB*3 and *ctxB*7 have been associated with the major cholera outbreaks worldwide (Kim *et al.*, 2015).

Recent studies revealed the *V. cholerae* O1 ET associated with cholera outbreaks in Haiti to be genetically closely related to ET strains associated with cholera in Asia (Hendriksen *et al.*, 2011). Based on single nucleotide polymorphism (SNP) analysis of the *V. cholerae* genome, the seventh cholera pandemic was shown to be transmitting worldwide via three major overlapping waves from the Ganges Delta of the Bay of Bengal, the historical home of Asiatic cholera (Muteurja *et al.*, 2011). Of the 13 recognized *ctxB* genotypes, *ctxB*3 was found associated with wave 1, *ctxB*1 with wave 2 and in early wave 3 strains; and *ctxB*7 was found only in the wave 3 strains, such as the 2010 Haiti cholera outbreak strains (Kim *et al.*, 2015).

The *V. cholerae* ET biotype (*ctxB*3) initiating the seventh cholera pandemic was replaced in Asia and Africa with *V. cholerae* ET carrying *ctxB*1 starting in 2001 (Nair *et al.*, 2006). This emergence and spread of altered-ET in Asia and Africa had huge epidemiological implications, as ET carrying *ctxB*1 was associated with more severe disease (Siddique *et al.*, 2010). Further change in the CTX prophage gene (histidine to asparagine at the twentieth position of *ctxB*) resulting in substitution of *ctxB*1 with *ctxB*7 was reported in 2006 from India (Naha *et al.*, 2012). A similar change from *ctxB*1 to *ctxB*7 also occurred in *V. cholerae* O1 responsible for endemic cholera in Dhaka, Bangladesh, but not before 2008 (Rashed *et al.*, 2012), suggesting a possible transmission of *V. cholerae* carrying the *ctxB*7 from neighbouring India, which was detected later in Africa and Haiti (Hasan *et al.*, 2012). Here, in the present study, we report yet another temporal change in the *ctxB* genotype of the *V. cholerae* population, showing that the *ctxB*1 returned and re-established dominance in 2013–2014, outcompeting *ctxB*7, which was dominant in the preceding years in *V. cholerae* associated with endemic cholera in Dhaka, Bangladesh (Rashed *et al.*, 2012).

*V. cholerae* was isolated from the stools of cholera patients admitted at the icddr,b Dhaka Hospital (Dhaka, Bangladesh); and the environmental isolates were from water samples routinely collected every fortnight from the water bodies in and around Dhaka city. A total of 151 conveniently isolated *V. cholerae* (70 clinical and 81 environmental) isolated between 2010 and 2011 were subjected to DNA sequencing using primers and conditions as described elsewhere (Olsvik *et al.*, 1993). The *ctxB* sequencing data (GenBank accession nos KT278766–KT278777) of the respective genotype, *ctxB*1 or *ctxB*7, were found to be consistent with the MAMA and DMAMA-PCR results. The *ctxB* genotyping data revealed that all *V. cholerae* O1 strains isolated from cholera cases had *ctxB*7 in 2010 (100 %; 20/20) and 2014 (100 %; 20/20), as reported earlier (Rashed *et al.*, 2012). Although the number of *V. cholerae* clinical strains included in 2012 was low (n=10), the majority of these strains (60 %; 6/10) carried *ctxB*1, and the *ctxB*7 was detected from the remaining *V. cholerae* associated with endemic cholera in Dhaka, Bangladesh. Subsequently, the proportion of *V. cholerae* carrying *ctxB*7 declined further and all *V. cholerae* tested from clinical sources carried *ctxB*1 in 2013 (100 %; 20/20) and 2014 (100 %; 20/20) (Fig. 1a). *V. cholerae* isolated from natural surface waters revealed the co-existence of the two *ctxB* genotypes, either *ctxB*1 or *ctxB*7 being present; the relative abundance of *ctxB*1 was more than half in 2010 (56 %; 9/16) and 2011 (55 %; 11/20) (Fig. 1b). While the basis for the temporal selection of *ctxB* genotype in *V. cholerae* responsible for endemic cholera is yet to be understood, *V. cholerae* O1 isolated from cholera patients carried *ctxB*7, not *ctxB*1, during 2010–2011 (Rashed *et al.*, 2012). In 2012, *V. cholerae* carrying *ctxB*1 biotype-specific toxin co-regulated pilus (*tcpA*<sup>ET</sup>), haemolysin (*hlyB*<sup>ET</sup>), phage repressor *rstR*<sup>ET</sup>, and repeat-in-toxin (*rxtC*) (Rivera *et al.*, 2003; Kimsey *et al.*, 1998; Chow *et al.*, 2001).
is that the SNPs of V. cholerae 2014). The limitation of the present study et al. 2012; Rashed et al. 2013; Alam et al. 2013, 2014). In this study, a significant correlation was observed between the clinical and environmental strains carrying either ctxB1 or ctxB7 (Spearman’s correlation coefficient=0.973; P=0.005). Although the epidemiological significance of the change from ctxB7 to ctxB1, as observed in Bangladesh during 2013–2014, is not well understood, the competition of the ctxB genotypes and their temporal shift in V. cholerae responsible for cholera at different ecological settings provide in situ evidence of CTX prophage-mediated evolution of the bacterium (Naha et al., 2012; Rashed et al., 2013; Alam et al., 2014). The limitation of the present study is that the SNPs of ctxB were monitored, not the genome-wide SNPs that have been proposed to drive positive selection of V. cholerae in Haiti (Azarian et al., 2014); and so, the synergistic role in the observed genotypic shift of SNPs elsewhere in the V. cholerae genome cannot be ruled out. In conclusion, the observed temporal fluctuation of ctxB genotypes in V. cholerae responsible for endemic cholera in Bangladesh suggests a possible role of CTX prophage in the natural selection and short-term evolution of the bacterium. The observed genotypic shift from ctxB7 to ctxB1 occurred after a couple of years of dominance of the former in Bangladesh. It is difficult to predict how the changing ctxB genotype is going to influence the epidemiology of cholera in this region. Nonetheless, the results presented in this study may have implications in understanding the short-term evolution of V. cholerae and in designing intervention and preventive measures against the bacterium responsible for the deadly disease cholera.

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