Short Report: A Cholera Outbreak of the *Vibrio cholerae* O1 El Tor Variant Carrying Classical *CtxB* in Northeastern Thailand in 2007

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Abstract. Cholera outbreaks occurred in Thailand in 2007. Isolates from the northeastern regions were analyzed. Interestingly, the outbreak strain was identified as biotype El Tor; serotype Ogawa with cholera toxin B subunit gene (*ctxB*) of the classical type and CTX prophage repressor gene of the El Tor type. The clone was genetically closely related to pulsotype H, which is predominantly found in India. It was probably introduced into Thailand recently.

An unusually high incidence of cholera cases was recorded in 50 provinces in Thailand in 2007.^{1–3} In the northeastern regions, the highest incidence of cholera cases occurred from September to November. In this study, *Vibrio cholerae* O1 was isolated from 10 patients in the Udonthani (four samples) and Khon Kaen (six samples) provinces during the outbreak (Figure 1). It was identified by biochemical tests and its agglutination with antiserum to *V. cholerae* O1 (Denka Seiken, Tokyo, Japan). The isolates were bityped by the Voges-Proskauer (VP) test and susceptibility to polymyxin B (50 units).^{4,5} Their susceptibility to 11 antibiotics (Becton Dickinson, Sparks, MD) was tested using a standard disc diffusion technique, according to the Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines.⁶ Virulence-associated genes and biotype markers in the genome were amplified using polymerase chain reaction (PCR) primers, as described previously.^{7–10}

The strains were genotyped using enterobacterial repetitive intergenic consensus (ERIC)-PCR, ribotyping, and pulsed-field gel electrophoresis (PFGE). The ERIC-PCR was performed using the ERIC1R and ERIC2 primers.¹¹ Ribotyping was performed as described previously.¹² The PFGE conditions were as described in the Pulsenet (www.cdc.gov/pulsenet) standardized protocol, with some modifications.

All of the isolates were identified as the *V. cholerae* O1, serotype Ogawa. The polymyxin B sensitivity and VP tests indicated that the isolates belonged to the biotype El Tor (Table 1).

The PCR analysis of the *rtxC* gene also revealed that these isolates were of the El Tor biotype,⁸ whereas a hexaplex PCR assay confirmed that a complete set of genes was present in all the isolates and gave positive results for the El Tor-specific *tcpA* gene. However, mismatch amplification mutation assay (MAMA)-PCR showed all the isolates carried the classical type *ctxB* gene (*ctxB*^{Cl}) (Table 1). To confirm our MAMA-PCR results, we sequenced the *ctxB* gene of the isolates. The deduced amino acid sequence of these strains was identical to that of the classical reference strain (569B), with histidine at position 39 and threonine at position 68 (data not shown). However, screening by PCR assays for the allele-specific CTX

prophage repressor gene (*rstR*)⁷ revealed that all the strains only produced amplicons of the El Tor type *rstR* (*rstR*^{El}). These experiments indicated that these isolates exhibited the El Tor genome backbone, but carried *ctxB*^{Cl}. *V. cholerae* O1 El Tor carrying *ctxB*^{Cl} (or CT1)^{13,14} is specifically called the El Tor variant or hybrid biotype.¹⁵

The ERIC-PCR of the genomic DNA of the *V. cholerae* strains resulted in the amplification of multiple DNA fragments of 0.6 to 10.0 kb (Figure 2). All the fragments were identical in all of the *V. cholerae* isolates. The ribotyping and PFGE patterns were also identical among all the Thai isolates.

We next compared the isolates' genotype with that of the major pulsotype found in India in 2004–2005. The H pulsotype was identified in India in July 1993 and was dominant among the *V. cholerae* O1 isolates in India in 2004–2005.^{16–19} The PFGE pattern between the Thailand and India strains showed a slight difference in one band (~190 kb), which was smaller in the H pulsotype, J16173 strain (Kolkata, India, lane 11) (Figure 2). However, by ERIC-PCR and ribotyping the strains were identical, indicating they were genetically closely related clones.²⁰

Antibiotic susceptibility testing showed that the 10 isolates were susceptible to chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), and gentamicin (10 µg), resistant to sulfamethoxazole/trimethoprim (SXT) (25 µg), tetracycline (30 µg), streptomycin (5 µg), polymyxin B (300 U), furazolidone (100 µg), and doxycycline (30 µg), and intermediate to ampicillin (10 µg). The O1 isolate in Kolkata, India, 2004 to 2005, is resistant to multiple antibiotics (ampicillin, SXT, furazolidone, nalidixic acid, and streptomycin) with a reduced susceptibility to ciprofloxacin. An increased isolation of tetracycline-resistant strains in India was noted in 2005 in India.²¹ Overall, the Indian and Thai isolates gave similar drug susceptibility patterns.

The Udonthani and Khon Kaen isolates showed the same genotypic and phenotypic traits, indicating that a single clone was disseminated throughout the outbreak in 2007. Furthermore, *V. cholerae* O1 isolated in Lamphun, northern Thailand during the same period showed the same phenotypic and genotypic traits (data not shown). Thus, the clone was not only disseminated in the northeastern regions, but also in at least one northern region.

Tapchaisri and others^{22,23} reported the genotypic characterization by PFGE and ribotyping of 240 *V. cholerae* isolates from patients with choleric diseases during outbreaks in March 1999–April 2000 and December 2001–February 2002

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FIGURE 1. Map of Thailand and locations of the provinces from where *Vibrio cholerae* O1 were isolated from patients.

in Thailand. Among them, 17 PFGE and 5 ribotyping patterns were recognized, indicating clonal diversity. The 2007 isolates of serotype Ogawa exhibited different ribotyping patterns from those of the past isolates. Therefore, they were probably introduced into Thailand in recent years.

V. cholerae O1 variants of the El Tor biotype spread to Asian and African countries,^{24–28} but have never been reported in Thailand. El Tor variants with *ctxB*^{Cl} and *rstR*^{El} were recently reported in Northern Vietnam, in outbreaks from late 2007 to early 2008,²⁹ whereas 12 isolates between 1995 and 2004 were identified as *V. cholerae* O1 biotype El Tor carrying *ctxB*^{Cl} and *rstR*^{Cl}. Recent Kolkata isolates including the J16173 strain also carry *ctxB*^{Cl} and *rstR*^{El}.^{15,17,18} We first reported in late 2007 that recent epidemic isolates of *V. cholerae* in northeastern Thailand are El Tor variant carrying *ctxB*^{Cl} and *rstR*^{El}. The clone causing the outbreak in Thailand may be one of these variants that spread to Thailand.

In this study, a cholera outbreak in Thailand in 2007 was found to be caused by a clone that was genetically related to the pulsotype H strains, which are dominant in India. It remains to be determined whether there was a sudden upsurge in cases of *V. cholerae* O1 infection in Thailand in 2007. However, it is well known that the outbreak in the northeastern regions of Thailand was caused by eating undercooked cockles, a type of shellfish.¹ We hypothesize that the epidemic clone transited to Thailand by the Andaman Sea and disseminated inland into Thailand. Understanding the transmission routes of toxigenic *V. cholerae* across countries may aid the development of countermeasures against cholera in Thailand and other countries.

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TABLE 1
Characterization of Thai *Vibrio cholerae* O1 strains used in this study*

Test strain origin (no. examined)	Years of isolation	Polymyxin B (50 U)	VP-test	PCR amplicons							
				<i>tcpA</i>	<i>ctxA</i>	<i>ctxB</i>	<i>zot</i>	<i>ace</i>	<i>toxR</i>	<i>rtxC</i>	<i>rstR</i>
Udonthani (4)	2007	Resistant	+†	El	+	Cl‡	+	+	+	+	El
KhonKaen (6)	2007	Resistant	+	El	+	Cl	+	+	+	+	El
Lamphun (1)	2007	Resistant	+	El	+	Cl	+	+	+	+	El
Reference strains											
569B (Classical)	1948	Sensitive	–	Cl	+	Cl	+	+	+	–	Cl
N16961 (El Tor)	1971	Resistant	+	El	+	El	+	+	+	+	El

*VP = Voges-Proskauer test; PCR = polymerase chain reaction.

†+ = positive; – = negative.

‡Cl = Classical allele; El = El Tor allele.

FIGURE 2. DNA fingerprints of *Vibrio cholerae* O1 generated by (A) enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) amplification, (B) ribotyping (*Bgl*II digestion), and (C) pulsed-field gel electrophoresis (*Not*I digestion). Molecular marker sizes are indicated on the left. Lanes: 1 to 4, *V. cholerae* O1 strains (Udonthani); Lanes: 5 to 10, *V. cholerae* O1 (Khon Kaen); Lane: 11, J16173 (Kolkata, India); Lane: 12, CE 87 (Kolkata, India).

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