Technical Appendix

Primers for 5 variable number of tandem repeat loci in Vibrio cholera:

Gene VC0147	Basepair 136981 137461	Primer (5' \rightarrow 3') TTGTCATGGCTTGGATTTGG TGTCGATCACCAATGGCTGC
VC0436-7	466861 467401	CGTGGTACTAAGTTCCACGC CGTTTTTACCACGCTCCGCTTC
VC1650	1778221 1778701	CTACCAAGCGGCGGTTAAGCTG TGGGCAACCTGCTGGTAGC
VCA0171	187561 188101	GCATCATCCACAGCGTTTGG GCTGAAGCCTTTCGCGATCC
VCA0283	303781 304201	GTACATTCACAATTTGCTCACCC ACTTCAAAACTATTGCGCAC

Standard conditions were a 30- μ L reaction containing 100 μ mol/L of each dNTP, 1.5 mmol/L MgCl₂, 1× PCR buffer (Invitrogen, Carlsbad, CA, USA), and 1 unit of Taq DNA polymerase (Invitrogen); cycling was 30 times at 95°C for 30 s, annealing (at 50°C or 55°C) for 30 s and 72°C for 30 s.

ClustalX (1) alignment of 6 sequences derived from *Vibrio cholerae* isolates to determine distinct alleles. Each row represents a single isolate. Each base is a different color. The repeating unit is the hexamer: AACAGC. The dashes mark bases missing relative to the longest repeat in the first row.



Row 1 = allele 1; rows 2, 3, and 4 = allele 2; rows 5 and 6 = allele 3

Distribution of sequence types by serotype and source in Bakerganj and Mathbaria. For each sequence type, the source is indicated by a C for clinical or an E for environmental, the number of isolates follows after n=, and the month(s) when it was isolated. The arrows indicate sequence types found at both locales, while the barbells indicate sequence types found in both clinical and environmental samples within a locale.

Bakerganj		Mathbaria
O1 Inaba	C $3,5,2,20,7$ n=1 Apr-04 C $3,5,2,1,7$ n=1 Apr-04 C $3,5,2,2,6$ n=2 Jun-04 E $3,4,2,1,12$ n=13 Sep 04 E $3,5,2,15,12$ n=1 Sep 04 E $3,5,2,20,12$ n=1 Sep 04 C $3,5,2,16$ n=5 Sep Oct Nov 04 C $3,5,2,1,6$ n=5 Sep Oct Nov 04 C $3,5,2,1,6$ n=1 Oct 04 C $3,5,2,1,5$ n=5 Oct 04 C $3,5,2,1,5$ n=5 Oct 04 C $3,5,2,1,5$ n=2 Nov 04 C $4,5,2,3,6$ n=1 Oct 04 C $3,5,2,3,6$ n=1 Dec 04 C $3,5,2,3,7$ n=1 Dec 04 C $3,5,2,2,7$ n=7 Feb-04 Apr 05 E $3,5,2,2,6$ n=1 Apr 05	C 3,5,2,2,14 n=3 Apr 04 C 2,5,2,2,6 n=6 Apr May 04 C 3,5,2,2,16 n=4 Apr May 04 C 3,5,2,2,7 n=1 May 04 C 2,5,2,18,6 n=1 May 04 C 2,5,2,17,6 n=1 May 04 E 3,5,2,2,7 n=12 Dec 04
01	C 1,1,3,8,8 n=1 Jul 04 C 1,1,3,9,8 n=4 Jul Aug Oct 04 C 6,1,3,8,8 n=2 Dec 04	C 4,2,2,7,6 n=1 Apr 04 C 5,2,3,10,8 n=1 Apr 04 C 1,1,3,9,8 n=1 Apr 04
Ogawa	C 2,1,3,7,8 n=1 Dec 04 C 2,1,3,8,7 n=1 Dec 04 C 5,1,3,8,8 n=1 Dec 04 E 2,1,3,8,8 n=1 Dec 04 E 2,1,3,8,8 n=1 Dec 04 Apr 05	C 2,1,4,10,4 n=1 Apr 04 C 5,1,4,10,4 n=1 Apr 04 C 1,1,3,6,7 n=2 Nov 04
O139	None	E 4,1,1,1,1 n=5 Mar Jul Sep 04 E 4,1,1,2,1 n=17 Sep 04 Apr 05 E 4,1,1,2,8 n=1 Apr 05

C: clinical isolate; E: environmental isolate

• indicates genotypes are found in both clinical and environmental isolates

: indicates genotypes found in both Bakerganj and Mathbaria