Supplementary material:

### Table 1: Dataset summary for CTA and CTB sequences from various serogroups

| Serogroups | Number of sequences | Biotypes | CTA | CTB |
|------------|---------------------|----------|-----|-----|
| O1         |                     | O1 El Tor | 8   | 113 |
|            |                     | O1 Classical | 6   | 3   |
|            |                     | O1 Matlab | 0   | 5   |
| O139       |                     |          | 5   | 37  |
| Non O1/O139 |                   |          | 8   | 7   |
| Total      |                     |          | 27  | 165 |

### Table 2: Mutations in CTA sequences among different serogroups as summarized from MSA.

| Strain | Serogroup | Biotype/Serotype | Length | No. of mutated residues | Mutation | Accession number |
|--------|-----------|------------------|--------|-------------------------|----------|------------------|
| 4260B  | O139      |                  | 46     | 1                       | I222Y    | CAA53975         |
| B      |           |                  | 188    | 2                       | R7W, E112G | AAR29797         |
| J31W   |           |                  | 258    | 3                       | S28N, V134G, G163R | ACU00910 |
| 203-93 | non       | O141             | 258    | 1                       | S28N     | AAL69945         |
| 571-88 | O1/O139   | O115             | 258    | 1                       | S28N     | AAL69944         |
| 1322-69| O37       |                  | 258    | 1                       | S28N     | AAL60525         |
| S7     |           |                  | 258    | 1                       | S28N     | BAA06288         |

### Table 3: Mutations in CTB sequences among different serogroups as summarized from MSA.

| Strain | Serogroup | Biotype/Serotype | Length | No. of mutated residues | Mutation | Accession |
|--------|-----------|------------------|--------|-------------------------|----------|----------|
| N 16961| O1        | El Tor           | 124    | 2                       | H18Y,T47I | AAF94613 |
| 2125   | O1        | El Tor           | 124    | 2                       | H18Y,T47I | CAA45193 |
| 169/12 | O1        | El Tor           | 123    | 1                       | H13P     | ACH70469 |
| 204/12 | O1        | El Tor           | 123    | 1                       | H13P     | ACH70470 |
| 319/03 | O1        | El Tor           | 123    | 3                       | H13P, H18Y, T47I | ACH70468 |
| 337/01 | O1        | El Tor           | 123    | 1                       | H13P     | ACH70472 |
| 354/02 | O1        | El Tor           | 123    | 3                       | H13P, H18Y, T47I | ACH70467 |
| 365/2 | O1        | El Tor           | 124    | 1                       | F25L     | ACF35009 |
| 366(1) | O1        | El Tor           | 124    | 1                       | F25L     | ACF35007 |
| 6732/80| O1       | El Tor           | 124    | 1                       | F25L     | ACF35008 |
| 120186 | O1       | El Tor           | 124    | 1                       | F25L     | ACF35006 |
| BX 330286|       | El Tor         | 124    | 1                       | F25L     | ACF35005 |
| Cis 77 | O1        | El Tor           | 124    | 1                       | F25L     | ACF35010 |
| Peru-044|        | El Tor          | 115    | 2                       | H18Y,T47I | ACH70463 |
| Peru-130|         | El Tor          | 123    | 2                       | H18Y,T47I | ACH70464 |
| Peru-296|         | El Tor          | 123    | 2                       | H18Y,T47I | ACH70456 |
| KSQLM03 | O1       | El Tor           | 123    | 3                       | H18Y,T47I, S60L | ABV74277 |
| KSQLM04 | O1       | El Tor           | 123    | 1                       | S60L     | ABV74281 |
| KSQLM05 | O1       | El Tor           | 123    | 2                       | H18Y,T47I | ABV74273 |
| KSQLM10 | O1       | El Tor           | 123    | 2                       | H18Y,T47I, S60L | ABV74278 |
| KSQLM11 | O1       | El Tor           | 123    | 3                       | H18Y,T47I, S60L | ABV74282 |
| KSQLM16 | O1       | El Tor           | 123    | 1                       | S60L     | ABV74283 |
| KSQLM21 | O1       | El Tor           | 123    | 3                       | H18Y,T47I, S60L | ABV74274 |
| KSQLM22 | O1       | El Tor           | 123    | 3                       | H18Y,T47I, S60L | ABV74284 |
| KSQLM48 | O1       | El Tor           | 123    | 2                       | H18Y,T47I | ABV74285 |
| KSQLJ05 | O1       | El Tor           | 123    | 2                       | H18Y,T47I | ABV74280 |
| KSQL337/01 | O1    | El Tor       | 123    | 2                       | H18Y,T47I | ABV74276 |
| KSQL3280/02 | O1  | El Tor        | 123    | 2                       | H18Y,T47I | ABV74275 |
| KSQL20/03 | O1   | El Tor         | 123    | 1                       | V52G     | ABV74279 |
| B65     | O1       | Mozambique      | 104    | 2                       | H18Y,T47I | AAY54184 |
| MG116226| O1      | Matlab          | 104    | 2                       | H18Y,T47I | ABG56897 |
| MG116025| O1      | Matlab          | 104    | 2                       | H18Y,T47I | ABG56881 |
| 1854    | O139     |                  | 124    | 2                       | H18Y,T47I | BAA06291 |
| 1854    | O139     |                  | 124    | 2                       | H18Y,T47I | ACF81827 |
| AK_31047| O139     |                  | 124    | 2                       | H18Y,T47I | ACF81828 |
| 1852    | O139     |                  | 124    | 2                       | H18Y,T47I | ACF81826 |
| MP_1950 | O139     |                  | 124    | 1                       | D7A      | ACF81829 |
| MP_2044 | O139     |                  | 119    | 1                       | D7A      | ACF81853 |
| NHCM297 | O139     |                  | 124    | 2                       | H13P, H18Y | ACF81847 |
| 2205769 | O139     |                  | 124    | 1                       | D7A      | ACF81837 |
Table 4: Mutations, their sequence positions, change in amino acid types, relative occurrence at surface, interface, interior regions of the complex in different serogroups is given.

| Mutation | Nature of Mutations | No. of Strains | CTA/CTB interface | Buried | Exposed | Strain(s) |
|----------|---------------------|----------------|-------------------|--------|---------|-----------|
| **O1/O139** |                      |                |                   |        |         |           |
| I222Y    | Non polar to Non polar | 1              |                   |        | ✓       | 4260B     |
| R7W      | Polar(-) to Non polar | 1              |                   |        | ✓       | B         |
| S28N     | Polar(0) to Polar(0) | 5              |                   |        | ✓       | J31W, 203-93, 571-88, 1322-69, S7 |
| E112G    | Non polar to Non polar | 1              |                   |        | ✓       | B         |
| V134G    | Non polar to Non polar | 1              |                   |        | ✓       | J31W      |
| G163R    | Non polar to Non polar(+) | 1          |                   |        | ✓       | J31W      |
| **CTB**  |                      |                |                   |        |         |           |
| **Non-O1/O139** |                  |                |                   |        |         |           |
| **D7A**  | Polar(-) to Non polar | 6              | ✓                 |        |         | MP1950, MP2044, 2205769, 2202931, 2203098, 737198 |
| H13P     | Polar(-) to Polar(0) | 6              | ✓                 |        |         | N16961, 2125, 319/03, 354/02, Peru044, Peru130, Peru296, Moz, KSMQ03, KSMQ05, KSMQ10, KSMQ11, KSMQ21, KSMQ22, KSMQ48, KSM05, KSZ337/01, KSZ280/02, MG116226, MG116025, 1854, AJ937, AK31047, AL1852, NHCM297, 577797, 4260B |
| H18Y     | Polar(-) to Non polar | 27             | ✓                 |        |         | N16961, 2125, 319/03, 354/02, Peru044, Peru130, Peru296, Moz, KSMQ03, KSMQ05, KSMQ10, KSMQ11, KSMQ21, KSMQ22, KSMQ48, KSM05, KSZ337/01, KSZ280/02, MG116226, MG116025, 1854, AJ937, AK31047, AL1852, NHCM297, 577797, 4260B |
| **F25L** | Non polar to Non polar | 6              | ✓                 |        |         | 365/02, 366/01, 6732/80, 120186, BXJ330286, Cis77 |
| **O1/O139** |                    |                |                   |        |         |           |
| **T47I** | Polar(0) to Non polar | 23             | ✓                 |        |         | N16961, 2125, 319/03, 354/02, Peru044, Peru130, Peru296, Moz, KSMQ03, KSMQ05, KSMQ10, KSMQ11, KSMQ21, KSMQ22, KSMQ48, KSM05, KSZ337/01, KSZ280/02, MG116226, MG116025, 1854, AJ937, AK31047, AL1852, NHCM297, 577797, 4260B |
| **CTB**  |                      |                |                   |        |         |           |
| **Non-O1/O139** |                  |                |                   |        |         |           |
| **Q3H**  | Polar(0) to Polar(+)  | 3              | ✓                 |        |         | KSMQ03, KSMQ04, KSMQ10, KSMQ11, KSMQ16, KSMQ21, KSMQ22 |
| **D7A**  | Polar(-) to Non polar | 3              | ✓                 |        |         | O26_63, O27_365/96, O44_506/94 |

ISSN 0973-2063 (online) 0973-8894 (print) 8 Bioinformation 6(1): 001-009 (2011) © 2011 Biomedical Informatics
Table 5: Function inference to known site directed mutants with corresponding relative occurrence at surface, interface, interior regions of the complex is given.

| Mutation | Inference | Reference | CTA/CTB interface | Buried | Exposed | Partially buried |
|----------|-----------|-----------|--------------------|--------|---------|------------------|
| R7K      | ADP-ribosyltransferase activity of CTA disrupted | Burnette et al. (1991), Hase et al. (1994), Chan et al. (2010) | [64,65,66] | ✓       |        |                  |
| R11K     | Reduced toxicity | Jobling & Holmes (2001) | ✓        |        |        |                  |
| I16A     | Reduced toxicity and enzymatic activity | Jobling & Holmes (2001) | ✓        | ✓      |        |                  |
| R25G     |            |          | ✓        | ✓      | ✓      |                  |
| E29H     | Reduced stability and toxicity | Tinket et al. (2003) | [68]     | ✓      |       |                  |
| CTA      | Y30W,A,H, S68Y + V72Y, E110D, E112D | | ✓        | ✓      | ✓      |                  |
| CTB      | R35D      | Reduced AB5 assembly | | ✓      | ✓      |                  |
| V46A     | Affects immunoreactivity | Jobling & Holmes (2002) | [69]     | ✓      |       |                  |
| G33E     | Over expressed CTB diminishes active CT production | Silva et al. (1998) | [70]     | ✓      | ✓      |                  |
| G33D     | Ablish receptor binding ability | Merritt et al. (1995) | [71]     | ✓      | ✓      |                  |
| CTB      | H57A      | Loss of toxicity | Aman et al. (2001) | [72]     | ✓      | ✓      |                  |
| L74D     | No AB5 formation | Tinket et al. (2003) | [68]     | ✓      | ✓      |                  |
| I77D     | No β5 assembly | | | ✓      | ✓      |                  |
| T78D     | No AB5 assembly | | | ✓      | ✓      |                  |