phenotypically confirmed ET strains amplified the primers for the *rtxC* gene which is unique to ET biotype, confirming their ET traits. In addition, all ET strains amplified the primers for $tcpA^{ET}$ and $rstR^{ET}$ genes, but not $tcpA^{CL}$ and $rstR^{CL}$ genes further confirming those strains to be that of ET biotype.

**Antibiotic Susceptibility Testing**

Response of *V. cholerae* O1 strains towards six different antibiotics revealed that multidrug resistant strains having 3-4 resistance markers were circulating between 2006 and 2011 (Table 1). . The drugs to which all of the 54 tested *V. cholerae* O1 strains showed resistance throughout the study period included sulphamithoxazole - trimethoprim (SXT) and furazolidone (FR); whereas resistance towards tetracycline (TE) and erythromycin (E) were highly unstable, varying between the years. All of the tested strains were uniformly susceptible towards gentamicin (CN) and ciprofloxacin (CIP). Three of nine *V. cholerae* O1 strains (33.3%) showing resistance to four drugs namely SXT, FR, TE, and E were found only during the year 2006. Year-wise data revealed that E resistance marker did not exist after it was last detected during 2006 until 2011 when one of nine (11%) *V. choleare* strains was resistant towards E. Our year-wise data also revealed that TE resistance in *V. choleare* causing cholera between 2006 and 2010 occurred among 33.3%, 77.8%, 0%, 100%, and 27.3% of the strains during 2006, 2007, 2008, 2009, and 2010 respectively; whereas all strains tested were sensitive to TE during the year 2011. Overall data suggest that continuous monitoring of drug response of *V. choleare* is of paramount therapeutic implication as far as the drug resistance markers are highly unstable and can change temporally making an effective drug ineffective.

**PCR assay for detecting SXT and class1 integron**

All drug resistant *V. choleare* strains (n=54) supported the amplification of the primers for gene SXT, a mobile genetic element carrying multi-drug resistance gene cassettes in bacteria. None of

the strains amplified the primers targeted for the genetic element *intI*1 in the present study, confirming the absence of class 1 integron among the tested *V. cholerae* O1 strains.

**_CtxB_ typing by MAMA-PCR**

All *V. cholerae* O1 strains, including the CL (O395) and ET (N16961) reference strains were analyzed by a specifically desinged PCR technique, MAMA-PCR to determine the CTX-B genotype. As shown in Table 1, *V. cholerae* O1 strains that were identified as ET, amplified the CL specific $ctxB^{CL}$ gene but not the $ctxB^{ET}$ gene, confirming the CTX genotype to be of the CL biotype. ET biotype strains possessing CL biotype-specific CTX genotype (genotype 1) confirmed all *V. cholerae* O1 strains to be hybrid ET that replaced the prototype 7$^{th}$ pandemic ET (genotype 3) in Dhaka, Bangladesh since 2001.

**Sequencing of _ctxB_ gene**

PCR-amplified *ctxB* genes (460bp) of representative strains from each year during 2006 – 2011 were sequenced and the deduced amino acid sequences were determined by employing bioinformatic tools comparing with the sequences of the classical and El Tor reference strains available in the GenBank (O395 and N16961). As shown in Figure 1, *V. choleare* isolated before 2008 showed the absolute amino acid sequence similarity with the classical biotype CT (*ctxB* genotype 1), having histidine and threonine at positions 39 and 68 respectively. However, an additional sequence variation was observed in position 20, where histidine usually occurring in CL and ET type CT was replaced by asparagine (H→N), in majority of strains isolated during 2008, and strains tested thereafter (Figure 1). The DNA sequence and the deduced amino acids matched with the new *ctxB* genotype (designated *ctxB* genotype 7) reported recently from cholera outbreak in Orissa, eastern India (Kumar *et al.*, 2009). Although, the *ctxB* genotype 7 was not found among three *V. choleare* altered ET strains representative of the year 2009 tested in the present study, all *V. choleare* strains tested thereafter (2010 – 2011) harbored the *ctxB* genotype 7 instead of *ctxB* genotype 1 in Dhaka, Bangladesh.

**PFGE analysis**

Representative *V. cholerae* O1 strains isolated between 2006 and 2011 were analyzed by pulsed-field gel electrophoresis (PFGE) to reveal the genetic relatedness of the multidrug resistant strains that were circulating in Dhaka city. The *NotI* restriction enzyme digested the genomic DNA into variable fragments and the fragment sizes ranged from 20 to 350 kb (Figure 2). *V. cholerae* strains having resistance markers for FR and SXT had identical banding pattern except that minor divergence was observed for a few strains. Other multi-drug resistant strains showing resistance toward FR, SXT and TE had closely related PFGE pattern, but not identical, suggesting genetic divergence (Figure 2). Cluster analysis by dendrogram (constructed by Dice similarity coefficient and UPGMA clustering method) of the PFGE images revealed that majority of the FR and SXT resistant strains share a closely related major cluster (cluster A), suggesting that they belonged to a single clonal lineage. Strains showing resistance towards FR, SXT, and TE varied in their PFGE patterns and did not belong to any specific cluster, suggesting clonal divergence.

## DISCUSSION

Clinically suspected cases of cholera are routinely treated with a 1-3 day course of effective antibiotics (Sack *et al.*, 2004; Saha 2006) with appropriate oral or intravenous fluid(s). However, clinical management of cholera and preventive measure against the disease is jeopardized because of rapid genetic divergence (Nair *et al.*, 2006, Kumar *et al.*, 2009) and emergence of multiple antibiotic resistant *Vibrio cholerae*, the cause of cholera (Glass *et al.*, 1980). Here we present data on phenotypic and genetic characteristics of *V. cholerae* O1 associated with epidemic cholera in Dhaka between 2006 and 2011 showing genetic transition and switching of *ctxB* allele from genotype 1 (CL) to genotype 7, including temporal variation in drug resistance, leading to the reversal of tetracycline (TE) sensitive *V. cholerae* that was resistant to this drug for many years in Bangladesh.

The results of microbiological culture, biochemical and serological tests primarily confirmed that all the tested *V. cholerae* strains belonged to serogroup O1. These primary

microbiological test results were complemented by simplex- and multiplex-PCR assays for *V. cholerae* species-specific gene *ompW*, virulence associated gene *ctxA* encoding the subunit 'A' of the CT, and the *wbe* gene encoding serogroup O1-spesific marker (Hoshino *et al.*, 1998), further confirming that the *V. cholerae* strains were toxigenic and belonged to serogroup O1 (Alam *et al.*, 2010). The presence of biotype specific marker genes such as *rtxC*, $tcpA^{ET}$, $rstR^{ET}$, and $ctxB^{CL}$ confirmed that the *V. cholerae* O1 strains causing endemic cholera in Dhaka, during 2006-2011 were ET but had *ctxB* of CL biotype, as reported previously (Nair *et al.*, 2006; Alam *et al.*, 2010).

Ever since effective antimicrobial agent was included as a successful therapeutic agent for the treatment of cholera (Greenough *et al.*, 1964; Lindenbaum *et al.*, 1967), TE had long been the drug of choice world-wide, except for young children and pregnant women (Greenough *et al.*, 1964, Sack *et al.*, 2004). Other effective drugs include FR, E, SXT, and chloramphenicol (C) (Greenough *et al.*, 1964). After a few years of successful use of these antimicrobial agents for treatment of cholera (Lindenbaum *et al.*, 1967), rapid emergence of *V. cholerae* resistant to antimicrobial agents was reported from Africa (Mhalu, *et al.*, 1979). Likewise, in December 1979, *V. cholerae* O1 resistant to TE, AMP, Kn, Sm, and SXT emerged as the cause of cholera at the Matlab Hospital, Bangladesh (Glass *et al.*, 1980). Subsequent studies have reported *V. cholerae* resistant to nalidixic acid (NA), and most recently to E and CIP. Higher MIC and clinical failures forced the clinicians to stop CIP, and choose azithromycin (AZM) as the only drug effective against cholera at the Dhaka Hospital of icddr,b. Recent studies on *V. cholerae* O1 associated with severely dehydrating cholera in South Western India over the past few years showed that *V. cholerae* are resistant to several antibiotics, including TE, FR, norfloxacin (NOR), and CIP (Jian *et al.*, 2011). In the present study, *V. cholerae* tested at the Dhaka Hospital of icddr,b were susceptible to CIP during 2006 - 2011, which suggests that CIP can now be an effective drug of choice for treatment of cholera. Although all *V. cholerae* tested were resistant to SXT and FR, and resistance to TE and E varied between 2006 and 2010, all tested *V. cholerae* were sensitive to TE during 2011, rendering it to be an effective drug of choice for treatment cholera, as reported earlier (Greenough *et al.*, 1964).

After multi-drug resistant *V. cholerae* first emerged (Mhalu *et al.*, 1979; Glass *et al.*, 1980, Glass *et al.*, 1983), the antibiotic susceptibility patterns of epidemic strains have changed frequently. Likewise, the emergence of *V. cholerae* O1 or O139 having resistance towards different drugs has been reported from Bangladesh (Faruque *et al.*, 1998). The genetic basis for such fluctuation in drug resistance was shown to be attributed to lateral acquisition of self transmissible genetic element designated SXT carrying multiple antibiotic resistance markers (Waldor, *et al.*, 1996). In the present study, a significant proportion of the tested *V. cholerae* strains, associated with cholera epidemic between 2006 and 2011 in Dhaka, Bangladesh were multi-drug resistant. Although, four different antibiotic resistance profiles were found, all tested *V. cholerae* strains had the SXT element, presumably in their genome; a result that appears in line with their consistent resistance towards SXT and FR. Temporal variation in the TE resistance was observed, as none of the tested strains isolated during the year 2008 and 2011 showed resistance to TE. TE resistance in *V. cholerae* is known to be plasmid mediated and vibrios do not stably maintain plasmids (Taneja *et al.*, 2010), which could be a reason for the temporal fluctuation in TE resistance and the temporal disappearance of this marker rendering it to be an effective drug of choice, as we have observed in this study during 2008 and 2011. Given that antibiotic susceptibility pattern of epidemic strains changes frequently, continuous monitoring of drug resistance in *V. cholerae* appears crucial for choosing an effective drug for the treatment of cholera.

Cholera toxin (CT), encoded by the *ctxAB* genes, is the major virulence factor responsible for severe diseases cholera, caused by *V. cholerae*. Divergence within the *ctxB* gene and the corresponding amino acid sequence was first reported in early 1990s, and based on amino acid substitutions at positions 39, 46, and 68, three different *ctxB* genotypes of *V. cholerae* O1 strains have been identified (Olsvik *et al.*, 1993). Genotype 1 is associated with strains of classical biotype worldwide and the US Gulf Coast, genotype 2 is found in pre-seventh pandemic El Tor biotype strains from Australia, and genotype 3 is found in El Tor biotype strains from the seventh pandemic and the Latin American epidemic (Olsvik *et al.*, 1993). However, subsequent investigation of the *ctxB* gene sequence revealed the presence of eleven distinct genotypes in different serogroups of *V. cholerae* (Marin *et al.*, 2011). Genotypes 1, 2, 3, 7, 10, 11 were found in serogroup O1 strains, genotypes 3, 4, 5, 6 were found in serogroup O139 strains, genotypes 7

and 8 were found only in serogroups O27 and O37 respectively (Marin *et al.*, 2011). A significant recent event in the history of cholera is the emergence of variant *V. cholerae* ET having *ctxB* gene of *V. cholerae* CL biotype (Nair *et al.*, 2006, Morita *et al.*, 2010), which has been recognized as a new pandemic pathogen, having the capacity to spread globally (Chin *et al.*, 2011, Nair *et al.*, 2002, Nair *et al.*, 2006) causing more severe disease (Siddique *et al.*, 2009). According to a recent study, a new *ctxB* variant of *V. cholerae* having an amino acid substitution at position 20 [Histidine (H)→Asparagine (N)], designated genotype 7, was found associated with a large cholera outbreak in Orissa, Eastern India (Kumar *et al.*, 2009). In the present study, we have confirmed the emergence of genotype 7 in Dhaka during 2008 and determined 2010 was the year of transition from *ctxB* genotype 1 to genotype 7 among the *V. cholerae* hybrid ET associated with endemic cholera in Dhaka between 2006 and 2011. Variant El Tor of *V. cholerae* O1 possessing *ctxB* genotype 7, as reported from India (Kumar *et al.*, 2009), showing reduced susceptibility to ciprofloxacin has been reported recently from Western Africa (Quilici *et al.*, 2010). *V. cholerae* strains with *ctxB* genotype 7 in Dhaka were uniformly sensitive towards ciprofloxacin, however, switching from *ctxB* genotype 1 to genotype 7 may be an yet another major turning point, assuming that the hybrid ET with the same *ctxB* genotype 7 was responsible for severe and devastating cholera epidemic that killed thousands of people in Haiti in 2010 (Chin *et al.*, 2011).

Pulsed-field gel electrophoresis (PFGE) has been a reliable molecular tool for typing enteric pathogens, including *V. cholerae*. Results of contemporary literatures on comparative genomic studies appear in concurrence with the PFGE-based conclusions of circulation of very closely related clone(s) of *V. cholerae* in the global wave of 7$^{th}$ pandemic cholera world-wide (Chun *et al.*, 2009; Chin *et al.*, 2011; Mutreza *et al.*, 2011). The overall PFGE results presented in this study, including the clustering of strains observed in dendrograms demonstrated high genetic relatedness of *V. cholerae* O1 strains that are associated with cholera in Dhaka city during 2006 - 2011. In the present study, clustering of *V. cholerae* O1 strains were highly correlated with the antibiotic resistance markers compared to the genetic change in the *ctxB* genotype, which is just a base substitution and unlikely to be reflected in the PFGE level. Nonetheless, the data presented in this study showed the transmission of varying antibiotic resistant but genetically very closely related *V. cholerae* O1 ET in Dhaka, Bangladesh. Moreover,

irrespective of the deduced genetic switching from *ctxB* 1 to *ctxB* 7, minor divergence in the banding pattern was observed in majority of strains, which showed varying resistance towards SXT, FR and TE, suggesting such minor divergence to be due to acquisition of mobile genetic elements (Chun *et al.*, 2009; Chin *et al.*, 2011; Mutreza *et al.*, 2011) carrying antibiotic resistance (Jesudason *et al.*, 1986).

Diarrhea continues to be one of the major causes of morbidity and mortality in many developing countries where population density is very high, safe drinking water is scarce and people do not have access to immediate healthcare facilities. Cholera, a climate-driven disease caused by toxigenic *V. cholerae* (Alam *et al.*, 2006), is the most severe of all diarrheas, which without treatment can kill the affected individuals within a few hours. In the changing climate, which will result major cities like Dhaka more densely populated due to influx of climate refugees, the hybrid variant of *V. cholerae* (Nair *et al.*, 2006; Chin *et al.*, 2011) is of increasing health concern for the growing population of Bangladesh because the deterioration of sanitation and hygiene and resulting fecal-oral infections can be more severe and difficult to manage clinically. Moreover, such severe infection caused by multi-drug resistant *V. cholerae* can result in higher case fatality rates, prolonged hospitalizations, more secondary infections, and increased health care costs. Our study revealed that ET biotype of *V. cholerae*, the most successful pandemic strain that had switched its cholera toxin since 2001 from ET type to CL type (hybrid ET) (Nair *et al.*, 2006), has undergone yet another subtle but remarkable change in its *ctxB* gene since 2008 in Dhaka, Bangladesh, as reported from India recently (Kumar *et al.*, 2009). The epidemiological significance of these genetic changes whether the increased severity caused by ET *V. cholerae* in recent years is due to switching of the *ctxB* gene from ET type to CL type, or from genotype 1 to genotype 7 is not fully understood. We speculate that these changes in the toxin type may be a factor related to the increasing severity of disease caused by hybrid ET during recent outbreaks (Siddique *et al.*, 2009; Goel *et al.*, 2010; Chin *et al.*, 2011). Finally, the data presented in this study on continued phenotypic and genetic changes underscore the need for continued monitoring of *V. cholerae* causing endemic cholera in Bangladesh, considering the increasing global burden of cholera, the origin and spread of new variants, especially because such changes can significantly influence the clinical management of cholera and its prevention.

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**Reference**

1. **Kaper, J. B.**, Morris, J. G Jr. & Levine, M. M. (1995). Cholera. *Clin Microbiol Rev* 8, 48–86.
2. **Dziejman, M.**, Balon, E., Boyd, D., Fraser, C. M., Heidelberg, J. F. & Mekalanos, J. J. (2002). Comparative genomic analysis of *Vibrio cholerae*: genes that correlate with cholera endemic and pandemic disease. *Proc Natl Acad Sci U. S. A.* 99, 1556–1561
3. **Woodward, W. E.** & Mosley, W.H (1972). The spectrum of cholera in rural Bangladesh – comparison of El Tor Ogawa and Classical Inaba infection. *Am J Epidemiol* 96(5), 342–351.
4. **Politzer, R** (1959). Cholera. Geneva, Switzerland: *World Health Organization*, pp. 11–50.
5. Cholera working group (1993). Large epidemic of cholera-like disease in Bangladesh caused by *Vibrio cholerae* O139 synonym Bengal. *Lancet* 342, 387-390.
6. **Ramamurthy, T.**, Garg, S., Sharma, R., Bhattacharya, S.K., Nair, G.B., Shimada, T., Takeda, T., Karasawa, T., Kurazano, H. & other authors (1993). Emergence of novel strain of *Vibrio cholerae* with epidemic potential in southern and eastern India. *Lancet* 341(8846), 703-4.
7. **Alam, M**, Hasan, N.A., Sadique, A., Bhuiyan, N.A., Ahmed, K.U., Nusrin, S., Nair, G.B., Siddique, A.K., Sack, R.B. & other authors (2006). Seasonal cholera caused by *Vibrio*

*cholerae* serogroups O1 and O139 in the coastal aquatic environment of Bangladesh. *Appl Environ Microbiol* 72(6), 4096-104.

8. **Sack, D.A.**, Sack, R.B., Nair, G.B. & Siddique, A.K. (2004). Cholera. *Lancet* 363(9404), 223-33.
9. **Lindenbaum,** J., Greenough, W.B. & Islam, M.R. (1967). Antibiotic therapy of cholera. *Bull World Health Organ* 36(6), 871-83.
10. **Jesudason, K.V.** & John, T.J. (1990). Transferable trimethoprim resistance of *Vibrio cholerae* O1 encountered in southern India. *Trans R Soc Trop Med Hyg* 39, 136–137.
11. **Maimone, F.**, Coppo, A., Pazzani, C., Ismail, S. O., Guerra, R., Procacci, P., Rotigliano, G. & Omar, K.H. (1986). Clonal spread of multiply resistance strains of *Vibrio cholerae* O1 in Somalia. *J Infect Dis* 153, 802–803.
12. **Faruque, A. S. G.**, Alam, K., Malek, M.A., Khan, M. G., Ahmed, S., Saha, D., Khan, W. A., Nair, G. B., Salam, M. A. & other authors (2007). Emergence of multidrug-resistant strain of *Vibrio cholerae* O1 in Bangladesh and reversal of their susceptibility to tetracycline after two years. *J Health Popul Nutr* 25, 241–243.
13. **Jain, M.**, Goel, A. K., Bhattacharya, P., Ghatole, M. & Kamboj, D. V. (2011). Multidrug resistant *Vibrio cholerae* O1 El Tor carrying classical *ctxB* allele involved in a cholera outbreak in South Western India. *Acta Trop* 117, 152-156.
14. **Kumar, P.**, Wilson, P. A., Bhai, R. & Thomas, S. (2010). Characterization of an SXT variant *Vibrio cholerae* O1 Ogawa isolated from a patient in Trivandrum, India. *FEMS Microbiol Lett* 303, 132-136.
15. **Quilici, M. L.**, Massenet, D., Gake, B., Bwalki, B. & Olson, D. M. (2010). *Vibrio cholerae* O1 variant with reduced susceptibility to ciprofloxacin, Western Africa. *Emerg Infect Dis* 16, 1804-1805.
16. **Nair,** G. B., Faruque, S. M., Bhuiyan, N. A., Kamruzzaman, M., Siddique, A. K. & Sack D. A (2002). New variants of *Vibrio cholerae* O1 biotype El Tor with attributes of the classical biotype from hospitalized patients with acute diarrhea in Bangladesh. *J Clin Microbiol* 40(9), 3296-9.
17. **Nair, G. B.**, Qadri, F., Holmgren, J., Svennerholm, A. M., Safa, A., Bhuiyan, N. A., Ahmad, Q. S., Faruque, S. M., Faruque, A. S. & other authors (2006). Cholera due to altered El Tor strains of *Vibrio cholerae* O1 in Bangladesh. *J Clin Microbiol* 44:4211–3.

18. **Safa,** A., Sultana, J., Cam P. D., Mwansa, J. C., Kong, R. Y (2008). *Vibrio cholerae* O1 hybrid El Tor strains, Asia and Africa. *Emerg Infect Dis* 14(6), 987-8.

19. **Siddique, A. K.,** Nair, G. B., Alam M., Sack, D. A., Huq, A., Nizam, A., Longini, I. M. Jr., Qadri, F., Faruque, S. M. & other authors (2010). El Tor cholera with severe disease: a new threat to Asia and beyond. *Epidemiol Infect* 138, 347-352.

20. **Chin, C. S.**, Sorenson, J., Harris, J. B., Robins, W. P., Charles, R. C., Jean-Charles, R. R., Bullard, J., Webster, D. R., Kasarskis, A. & other authors (2010). The origin of Haitian cholera outbreak strains. *N Engl J Med* 364, 33-42.

21. **Kumar, P.**, Jain, M., Goel, A. K., Bhadauria, S., Sharma, S. K., Kamboj, D. V., Singh, L., Ramamurthy, T. & Nair, G. B. (2009). A large cholera outbreak due to a new cholera toxin variant of the *Vibrio cholerae* O1 El Tor biotype in Orissa, Eastern India. *J Med Microbiol* 58, 234–238.

22. **Kimsey,** H. H., Nair, G. B., Ghosh, A. & Waldor, M. K. (1998). Diverse CTXphis and evolution of new pathogenic *Vibrio cholerae*. *Lancet* 352(9126), 457-8.

23. **Chow**, K.H., Ng, T. K., Yuen, K. Y. & Yam, W. C. (2001). Detection of RTX toxin gene in *Vibrio cholerae* by PCR. *J Clin Microbiol* 39, 2594–2597

24. **Alam, M.**, Sultana, M., Nair, G. B., Siddique, A. K., Hasan, N. A., Sack, R. B., Sack, D. A., Ahmed, K. U., Sadique, A. & other authors (2007). Viable but nonculturable *Vibrio cholerae* O1 in biofilms in the aquatic environment and their role in cholera transmission. *Proc Natl Acad Sci U S A* 104, 17801-17806.

25. **Nusrin, S.,** Gil, **A**. I., Bhuiyan, N. A., Safa, A., Asakura, M., Lanata, C. F., Hall, E., Miranda, H., Huapaya, B. & other authors (2009). Peruvian *Vibrio cholerae* O1 El Tor strains possess a distinct region in the Vibrio seventh pandemic island-II that differentiates them from the prototype seventh pandemic El Tor strains. *J Med Microbiol* 58, 342–354.

26. **Hoshino,** K., Yamasaki, S., Mukhopadhyay, A. K., Chakraborty, S., Basu, A., Bhattacharya, S. K., Nair, G. B., Shimada, T., & Takeda, Y. (1998). Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic *Vibrio cholerae* O1 and O139. *FEMS Immunol Med Microbiol* 20, 201-207.

27. **Morita, M.**, Ohnishi, M., Arakawa, E., Bhuiyan, N. A., Nusrin, S., Alam, M., Siddique, A. K., Qadri, F., Izumiya, H., Nair, G. B. & Watanabe, H. (2008). Development and

validation of a mismatch amplification mutation PCR assay to monitor the dissemination of an emerging variant of *Vibrio cholerae* O1 biotype El Tor. *Microbiol Immunol* 52, 314–7

28. **Olsvik, O.**, Wahlberg, J., Petterson, B., Uhlen, M., Popovic, T., Wachsmuth, I. K. & Fields, P. I. (1993). Use of automated sequencing of polymerase chain reaction–generated amplicons to identify three types of cholera toxin subunit B in *Vibrio cholerae* O1 strains. *J Clin Microbiol* 31, 22–5.
29. **Thungapathra, M.,** Thungapathra, M., Amita, Sinha, K. K., Chaudhuri, S. R., Garg, P., Ramamurthy, T., Nair, G. B. & Ghosh, A (2002). Occurrence of Antibiotic Resistance Gene Cassettes *aac(6')-Ib, dfrA5, dfrA12,* and *ereA2* in Class I Integrons in Non-O1, Non-O139 *Vibrio cholerae* Strains in India. *Antimicrob Agents Chemother* 46(9), 2948-2955.
30. **Alam, M.**, Nusrin, S., Islam, A., Bhuiyan, N. A., Rahim, N., Delgado, G., Morales, R., Mendez, J. L., Navarro, A., Gil, A. I., Watanabe, H., Morita, M., Nair, G. B. & Cravioto, A. (2010). Cholera between 1991 and 1997 in Mexico was associated with infection by classical, El Tor, and El Tor variants of *Vibrio cholerae*. *J Clin Microbiol* 48(10), 3666-74.
31. **Saha,** D., Karim, M. M., Khan, W. A., Ahmed, S., Salam, M. A. & Bennish, M. L (2006). Single-dose azithromycin for the treatment of cholera in adults. *N Engl J Med* 354(23), 2452-62
32. **Glass, R. I.**, Huq, I., Alim, A. R. M. A., Yunus, M. (1980). Emergence of multiple antibiotic-resistant *Vibrio cholerae* in Bangladesh. *J Infect Dis* 142, 939–42.
33. **Greenough, W. B. 3rd,** Gordon, R. S. Jr, Rosenberg, I. S., Davies, B. I. & Benenson, A. S. (1964). Tetracycline in the treatment of Cholera. *Lancet* 1(7329), 355-7.
34. **Mhalu,** F. S., Mmari, P. W. & Ijumba, J. (1979). Rapid emergence of El Tor *Vibrio cholerae* resistant to antimicrobial agents during first six months of fourth cholera epidemic in Tanzania. *Lancet* 1(8112), 345-7.
35. **Glass, R. I.**, Huq, M. I., Lee, J. V., Threlfall, E. J., Khan, M. R., Alim, A. R., Rowe, B. & Gross, R. J. (1983). Plasmid-borne multiple drug resistance in *Vibrio cholerae* serogroup O1, biotype El Tor: evidence of a pointsource outbreak in Bangladesh. *J Infect Dis* 147, 204–9.

36. **Faruque, A. S. G.**, Salam, M. A., Faruque, S. M. & Fuchs, G. J. (1998). Aetiological, clinical and epidemiological characteristics of a seasonal peak of diarrhoea in Dhaka, Bangladesh. *Scand J Infect Dis* 30(4), 393-6.

37. **Waldor, M. K.**, Tschape, H. & Mekalanos, J. J (1996). A new type of conjugative transposon encodes resistance to sulfamethoxazole, trimethoprim, and streptomycin in *Vibrio cholerae* O139. *J Bacteriol* 178, 4157–65.

38. **Taneja, N.**, Samanta, P., Mishra, A. & Sharma, M. (2010). Emergence of tetracycline resistance in *Vibrio cholerae* O1 biotype El Tor serotype Ogawa from north India. *Indian J Pathol Microbiol* 53, 865-866

39. **Marin, M. A.** & Vicente, A. C. (2011). Variants of *Vibrio cholerae* O1 El Tor from Zambia showed new genotypes of *ctxB*. *Epidemiology and Infection* 23, 1-2

40. **Goel, A. K.** & Jiang, S. C. Genetic determinants of virulence, antibiogram and altered biotype among the *Vibrio cholerae* O1 isolates from different cholera outbreaks in India. *Infect Genet Evol* 10, 815–9.

41. **Chun, J.**, Grim, C. J., Hasan, N. A., Lee, J. H., Choi, S. Y., Haley, B. J., Taviani, E., Jeon, Y. S., Kim, D. W. & other authors (2009).
Comparative genomics reveals mechanism for short-term and long-term clonal transitions in pandemic *Vibrio cholerae*. *Proc Natl Acad Sci U S A* 106(36), 15442-7

42. **Mutreja, A.**, Kim, D. W., Thomson, N. R., Connor, T. R., Lee, J. H., Kariuki, S., Croucher, N. J., Choi, S. Y., Harris, S. R. & other authors (2011). Evidence for several waves of global transmission in the seventh cholera pandemic. *Nature* 477(7365), 462-5

**Table1**. Phenotypic and molecular characteristics of *Vibrio cholerae* O1 El Tor causing endemic cholera in Dhaka, 2006 -2011

| Year of isolates | No. of strains | Serotype | *wbeO1* | PolyB (50U) | CCA | Phage IV | Phage V | *ctxA* | *RTX* | *rstR* | *tcpA* | *ctxB* Type | Antibiotic Resistance Profile | *sxt* | *intI1* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2006 | 6 | Ogawa | + | R | + | R | S | + | ET | ET | ET | CL | SXT, FR | + | - |
| | 3 | Ogawa | + | R | + | R | S | + | ET | ET | ET | CL | SXT, FR, TE, E | + | - |
| 2007 | 2 | Ogawa | + | R | + | R | S | + | ET | ET | ET | CL | SXT, FR | + | - |
| | 7 | Ogawa | + | R | + | R | S | + | ET | ET | ET | CL | SXT, FR, TE | + | - |
| 2008 | 8 | Ogawa | + | R | + | R | S | + | ET | ET | ET | CL | SXT, FR | + | - |
| 2009 | 8 | Ogawa | + | R | + | R | S | + | ET | ET | ET | CL | SXT, FR, TE | + | - |
| 2010 | 8 | Ogawa | + | R | + | R | S | + | ET | ET | ET | CL | SXT, FR | + | - |
| | 3 | Ogawa | + | R | + | R | S | + | ET | ET | ET | CL | SXT, FR, TE | + | - |
| 2011 | 8 | Ogawa | + | R | + | R | S | + | ET | ET | ET | CL | SXT, FR | + | - |
| | 1 | Ogawa | + | R | + | R | S | + | ET | ET | ET | CL | SXT, FR, E | + | - |
| 1975 | N16961 | Inaba | + | R | + | R | S | + | ET | ET | ET | ET | All Sensitive | - | - |
| 1965 | O395 | Ogawa | + | S | - | S | R | + | CL | CL | CL | CL | All Sensitive | - | - |

**Fig. 1.**

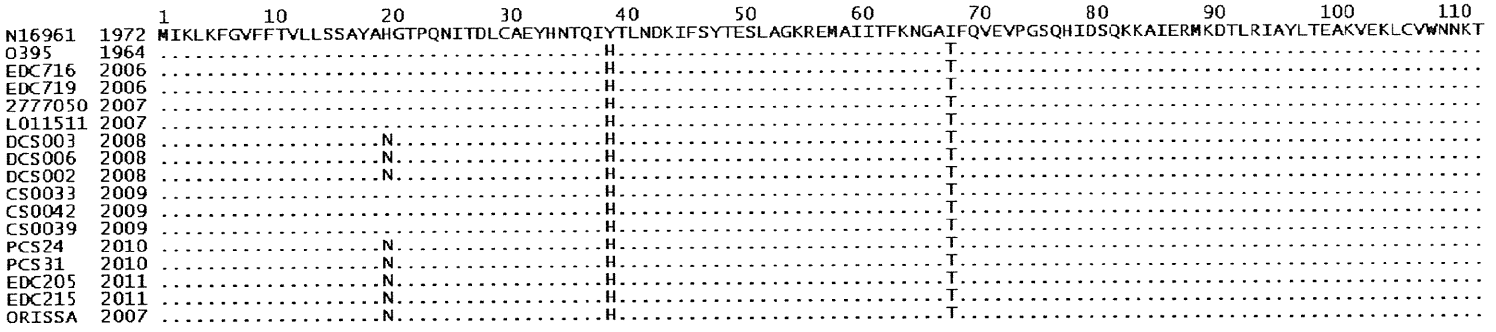


**Fig1:** Amino acid sequence alignment of the CTXB subunit of *V. cholerae* O1 El Tor strains isolated from Bangladesh (2006-2011) with El Tor reference (N16961), classical reference (O395) and Orissa variant (genotype 7). Identical amino acid residues are indicated by dots. The amino acid sequences of CTXB of reference and Orissa variant used in the alignment were taken from GenBank.

**Fig. 2**

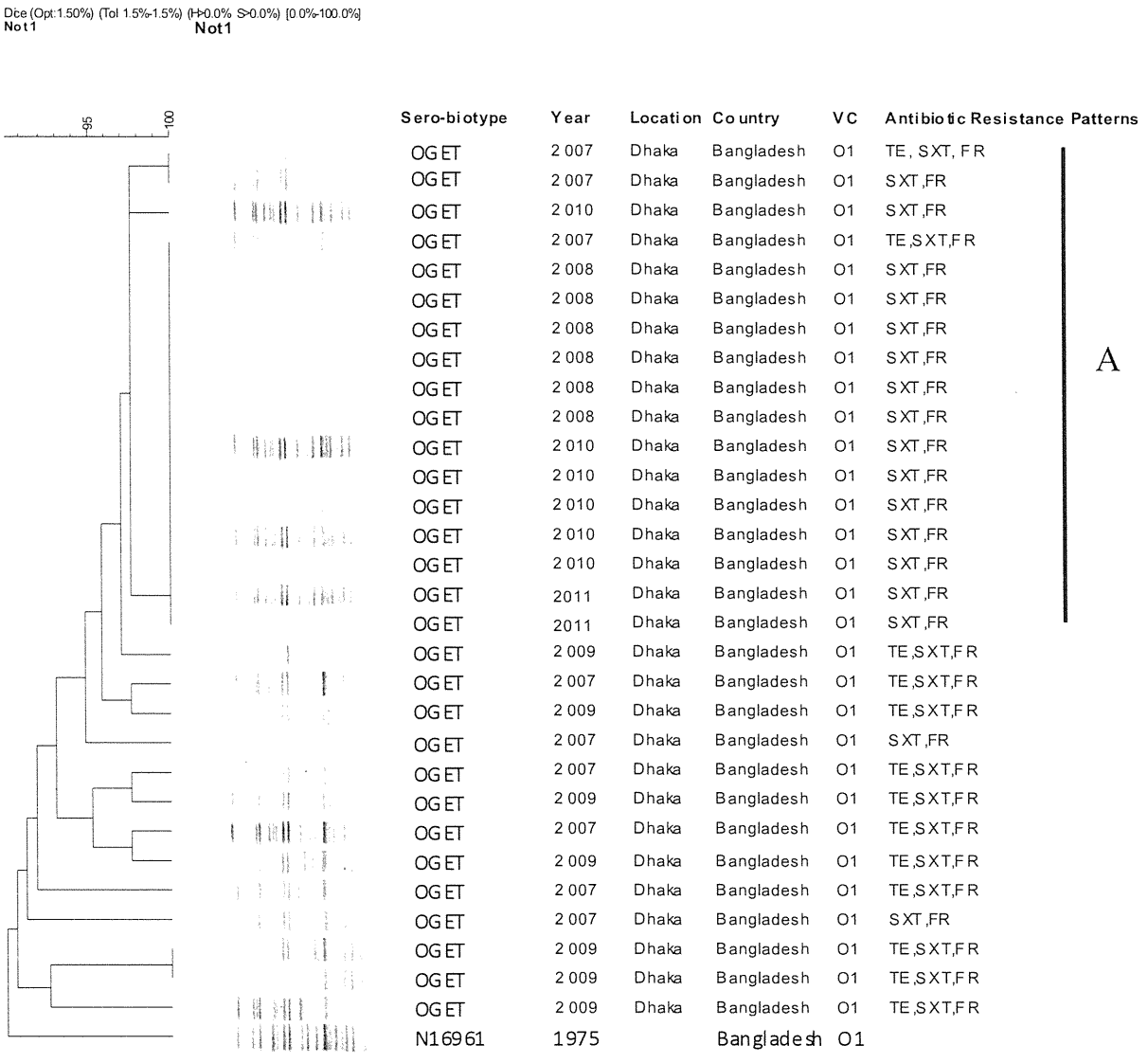


**Fig 2:** Molecular fingerprinting of drug resistant *V. cholerae* O1 El Tor strains isolated in Bangladesh between 2006 and 2011 determined by pulsed-field gel electrophoresis (PFGE) of *NotI*-restriction digested genomic DNA. Dendrogram was constructed from the PFGE patterns. Cluster analysis by dendrogram (UPGMA clustering method) showed majority of *V. cholerae* O1 ET biotype strains having resistance markers for SXT,FR shared a major cluster (cluster A) suggesting those to be clonal except minor divergence was observed for multi-drug resistant strains having $TE^R$ marker.

# 平成２３年度業績

＊研究成果の刊行に関する一覧表

＊学会発表一覧表

# 研究成果の刊行に関する一覧表　(平成２３年度)

| 執筆者氏名 | 刊行書籍又は雑誌名<br>（雑誌のときは雑誌名、<br>巻号数、論文名） | 刊行書店名 | 巻名 | ページ | 刊行年 |
|---|---|---|---|---|---|
| プロジェクト１：中国 | | | | | |
| Nakajima N, Kitamori Y, Ohnaka S, Mitoma Y, Mizuta K, Wakita T, Shimizu H, Arita M. | Development of a transcription-reverse transcription concerted reaction method for specific detection of human enterovirus 71 from clinical specimen | J Clin Microbiol | | (in press) | |
| Fujimoto T, Iizuka S, Enomoto M, Abe K, Yamashita K, Hanaoka N, Okabe N, Yoshida H, Yasui Y, Kobayashi M, Fujii Y, Tanaka H, Yamamoto M, Shimizu H | Hand, Foot, and Mouth Disease Caused by Coxsackievirus A6, Japan, 2011. | Emerg Infect Dis | 18 | 337-339 | 2012 |
| Konno M, Yoshioka M, Sugie M, Maguchi T, Nakamura T, Kizawa M, Umegaki Y, Yasutake H, Ishikawa Y, Hanaoka N, Okabe N, Taniguchi T, Shimizu H, Fujimoto T: | Fourteen years' surveillance of coxsackievirus group A in Kyoto 1996-2009 using mouse, RD-18S, and Vero Cells. | Jpn J Infect Dis | 64 | 167-168 | 2011 |
| Iwai M, Horimoto E, Obara M, Obuchi M, Kurata T, Kawagoshi K, Nakamura S, Shimizu H, Yoshida H, Takizawa T: | Endemic transmission of echovirus 30 in Toyama, Japan in 2010 is verified by environmental surveillance. | Jpn J Infect Dis | 64 | 165-167 | 2011 |
| Miyamura K, Nishimura Y, Abo M, Wakita T, and Shimizu H: | Adaptive mutations in the genomes of enterovirus 71 strains following infection of mouse cells expressing human P-selectin glycoprotein ligand-1 | J Gen Virol | 92: | 287-291, | 2011 |

| | | | | | |
|---|---|---|---|---|---|
| Shimizu H (分担執筆) | A Guide to Clinical management and Public Health Response for Hand Foot Mouth Disease (HFMD) | WHO report, | | | 2011 |
| Zhang Y, Wang J, Guo W, Wang H, Zhu S, Wang D, Bai R, Li X, Yan D, Zhu Z, Tan X, An H, Xu A, Xu W. | Emergence and transmission pathways of rapidly evolving evolutionary branch c4a strains of human enterovirus 71 in the central plain of china. | PLoS One | 6 | e27895 | 2011 |
| Tan X, Huang X, Zhu S, Chen H, Yu Q, Wang H, Huo X, Zhou J, Wu Y, Yan D, Zhang Y, Wang D, Cui A, An H , Xu W. | The persistent circulation of enterovirus 71 in People's Republic of China: causing emerging nationwide epidemics since 2008. | PLoS One | 6 | e25662 | 2011 |
| 西山明宏, 石田直, 興梠陽平, 小西聡史, 坪内和哉, 伊賀知也, 國政啓, 岩破将博, 福山一, 仲川宏昭, 伊藤明広, 生方智, 吉岡弘鎮, 橘洋正, 有田真知子, 橋本徹, 前川純子 | *Legionella pneumophila* serogroup3 による呼吸器感染症の 4 症例 | 感染症学雑誌 | 85 | 373-379 | 2011 |
| Fukushi S, Nakauchi M, Mizutani T, Saijo M, Kurane I, Morikawa S. | Antigen-capture ELISA for the detection of Rift Valley fever virus nucleoprotein using new monoclonal antibodies. | J Virol Methods. | | (in press) | |
| Nakayama, S., Tribuddharat, C., Prombhul, S., Shimuta, K., Srifuengfung, S., Unemo, M., and Ohnishi, M | Molecular analyses of TEM genes and their corresponding penicillinase-producing Neisseria gonorrhoeae isolates in Bangkok, Thailand. | Antimicrob. Agents Chemoter | 56 | 916-920 | 2012 |
| Otsuka N, Han HJ, Toyoizumi-Ajisaka H, Nakamura Y, Arakawa Y, Shibayama K, | Prevalence and genetic characterization of pertactin-deficient *Bordetella pertussis* in Japan. | PLoS ONE | 7(2) | e31985. | 2012 |

| | | | | | |
|---|---|---|---|---|---|
| Kamachi K. | | | | | |
| Suzuki T, Kataoka H, Ida T, Kamachi K, Mikuniya T. | Bactericidal activity of topical antiseptics and their gargles against *Bordetella pertussis*. | J Infect Chemother | | (in press) | |

プロジェクト２：台湾

| | | | | | |
|---|---|---|---|---|---|
| **Kobayashi, K.**, M. Ato, and S. Matsumoto | Global threats and the control of multidrug-resistant tuberculosis. | J. Disaster Res | 6 | 443-450 | 2011 |
| Takatsuka, M., M. Osada-Oka, E. F. Satoh, K. Kitadokoro, Y. Nishiuchi, M. Yoshimura-Niki, M. Inoue, K. Iwai, T. Arakawa, Y. Shimoji, H. Ogura, **K. Kobayashi**, A. Rambukkana, and S. Matsumoto. | A histone-like protein of mycobacteria possesses ferritin superfamily protein-like activity and protects against DNA damage by Fenton reaction. | PLoS One | 6 | e20985 | 2011 |
| Ozeki, Y., Y. Hirayama, T. Takii, S. Yamamoto, **K. Kobayashi**, and S. Matsumoto. | Loss of anti-mycobacterial efficacy in mice over time following vaccination with *Mycobacterium bovis* bacillus Calmette-Guérin. | Vaccine | 29 | 6881-6887 | 2011 |
| Naka, T., N. Nakata, S. Maeda, R. Yamamoto, M. Doe, S. Mizuno, M. Niki, **K. Kobayashi**, H. Ogura, M. Makino, and N. Fujiwara. | Structure and host recognition of serotype 13 glycopeptidolipid from *Mycobacterium intracellulare*. | J. Bacteriol. | 193 | 5766-5774 | 2011 |
| Naka, T., S. Maeda, R. M. Niki, N. Ohara, S. Yamamoto, I. Yano, J.-i. Maeyama, H. Ogura, **K. Kobayashi**, and N. Fujiwara. | Lipid phenotype of two distinct subpopulations of *Mycobacterium bovis* bacillus Calmette-Guérin Tokyo 172 substrain. | J. Biol. Chem. | 286 | 44153 -44161 | 2011 |
| Khin S. A., Matsuoka M, Kai M., Kyaw K., | FTA Card Utility for PCR Detection of *Mycobacterium leprae*. | Jpn J Infect Dis. | Vol. 64 | 246-248 | 2011 |

| | | | | | |
|---|---|---|---|---|---|
| Win A. A., Shwe M. M., Thein M., Htoo M. M., & Htoon M. T. | | | | | |
| Nakata N., Kai M., Makino M. | Amino acid substitutions at position 95 in GyrA can add fluoroquinolone resistance to *Mycobacterium leprae.* | Antimicrobial Agent Chemother. Doi: | 10.1128/AAC. | 05831-11 | 2012 |
| Li W., Matsuoka M., Kai M., Thapa P., Khadge S., Hagge D. A., Brennan P. J., Vissa V. | Real-time PCR and high resolution melt analysis for rapid detection of *Mycobacterium leprae* drug resistance mutations and strain types. | J Clin Microbiol. | Vol. 50 | 742-753 | 2011 |
| Otsuka N, Han HJ, Toyoizumi-Ajisaka H, Nakamura Y, Arakawa Y, Shibayama K, Kamachi K. | Prevalence and genetic characterization of pertactin-deficient *Bordetella pertussis* in Japan. | PLoS ONE | 7(2) | e31985 | 2012 |
| Suzuki T, Kataoka H, Ida T, Kamachi K, Mikuniya T. | Bactericidal activity of topical antiseptics and their gargles against *Bordetella pertussis.* | J Infect Chemother | | (in press) | |

## プロジェクト３：インド

| | | | | | |
|---|---|---|---|---|---|
| Mitobe J, Yanagihara I, Ohnishi K, Yamamoto S, Ohnishi M, Ishihama A and Watanabe H. | acterial cytoskeleton protein RodZ is involved in post-transcriptional processing of *Shigella* type III secretion system. | EMBO reports 12 | | 911-916 | 2011 |
| Khan, S. M., Debnath, C., Pramanik, A. K., Xiao, L., Nozaki, T., and Ganguly, S. | Molecular evidence for zoonotic transmission of Giardia duodenalis among dairy farm workers in West Bengal, India. | Vet Parasitol. | 178 | 342-345 | 2011 |
| Takahara Y, Matsuoka S, Kuwano T, Tsukamoto T, Yamamoto H, Ishii H, Nakasone T, Takeda A, Inoue M, Iida A, Hara H, Shu T, Hasegawa M, Sakawaki H, Horiike M, | Dominant induction of vaccine antigen-specific cytotoxic T lymphocyte responses after simian immunodeficiency virus challenge. | Biochem Biophys Res Commun | 408 | 615-619 | 2011 |