Jpn. J. Infect. Dis., 65, 358-360, 2012

Laboratory and Epidemiology Communications

Occurrence of Toxigenic *Vibrio cholerae* in Accessible Water Sources of Cholera Endemic Foci in India

Prasenjit Batabyal, Subham Mookerjee, and Anup Palit*

Division of Bacteriology, National Institute of Cholera & Enteric Diseases (Indian Council of Medical Research), Kolkata, India

Communicated by Makoto Ohnishi

(Accepted May 8, 2012)

Cholera outbreaks cause major public health problems in India. West Bengal, along the Ganges delta, is the ''homeland of cholera,'' with frequent localized outbreaks (1). *Vibrio cholerae*, the causative organism, is an autochthonous inhabitant of brackish water and estuarine systems (2). Among 206 O serogroups of *V. cholerae*, only the O1 and the later described O139 serogroups are capable of causing epidemic cholera (3). Irrespective of their toxin-producing ability, *V. cholerae* O1 and O139 serogroups are rarely found in natural aquatic environs. Toxigenic *V. cholerae* is isolated only infrequently from surface water during epidemic and interepidemic periods (4). The present study was undertaken to identify *V. cholerae* in potable water sources from different outbreaks as well as randomly chosen foci to clarify their role in transmission dynamics with water as a major transmission vehicle.

A very high incidence of cholera is reported in the city of Kolkata (5), which is considered a cholera endemic zone (6). Between February 2008 and August 2009, a total of 426 water samples from household sources, including tap, tube well, and stored, were collected from the same locality of an urban community setup in a slum dwelling in Kolkata, where the incidence rate of cholera has been found to be as high as 1.6/1,000/year, with the greatest burden in children under 5 years of age (5). The samples were categorized as outbreak samples, which were collected from houses where cholera outbreak had been reported or where residents with active episodes were present during the collection period, and as non-outbreak samples, which were collected from houses where no diarrheal outbreak had been encountered within the past 3 months. Samples were collected aseptically in sterile glass bottles and stored at 4°C for transport from the site of collection to the laboratory for analysis.

After physiochemical evaluation (pH and salinity), each sample was subjected to coliform load detection by filtration of 100 mL and 10 mL water (using 0.22-μm filter paper; Millipore, Billerica, Mass., USA) followed by inoculation of filter paper on Chromocult Coliform Agar (Merck, Darmstadt, Germany). After overnight incubation at 37°C, all pink and blue colonies were counted to determine the coliform load. Simultaneously, 100 mL of each sample was enriched using 10× con-

*Corresponding author: Mailing address: Division of Bacteriology, National Institute of Cholera & Enteric Diseases, P-33, Scheme-XM, CIT Road, Beliaghata, Kolkata-700 010, India. Tel: +91-33-2370-0448/5533/4478, Fax: +91-33-2370-5066, E-mail: palit_anup@rediffmail.com

Table 1. Zone wise distribution of *V. cholerae* O1 and non-O1/non-O139 in the study foci

Source	No. of *V. cholerae* isolates (%)	No. of *V. cholerae* O1 isolates (%)	No. of toxigenic *V. cholerae* O1 isolates (%)	No. of *V. cholerae* non-O1/non-O139 isolates (%)	Statistical analysis
Outbreak samples $n = 120$[1)]	27 (23)	20 (16.7)	9 (7.5)	7 (5.8)	RR = 3.18 (1.71 < RR < 5.94)
Non-outbreak samples $n = 306$[1)]	65 (21)	16 (5.2)	1 (0.3)	49 (16)	

[1)]: Total number of samples analyzed.
RR, relative risk.

centrated 10 mL alkaline peptone water (BD/DIFCO, Sparks, Md., USA). Afterwards, 5 μL of each of the enriched samples was streaked onto thiosulfate citrate bile salts sucrose agar (TCBS; BD/DIFCO) and incubated at 37°C for 18 to 24 h. Presumptive *Vibrio* colonies (yellow colonies with elevated centers) were subjected to biochemical tests to isolate the suspected *V. cholerae* from the other vibrios; subsequently the biochemically identified *V. cholerae* colonies were inoculated in nutrient agar (BD/DIFCO) and incubated overnight at 37°C. The next day the colonies from the nutrient agar were subjected to oxidase test using oxidase reagent (BD/DIFCO).

Biochemically confirmed *V. cholerae* isolates were subjected to a simplex PCR, targeting *ompW* (588 bp) genes specific for *V. cholerae* (7). *V. cholerae* isolates were confirmed for serogroup from a non-selective medium by slide agglutination using *V. cholerae* O1 Poly, Ogawa, and Inaba antisera (BD/DIFCO), and Bengal O139 antisera (Denka-Seiken, Tokyo, Japan). *V. cholerae* O1 isolates were subjected to mismatch amplification mutation assay based (MAMA) PCR to detect the nucleotide sequence difference at position 203 of the *ctxB* cholera toxin gene for the identification of Classical and El Tor biotypes (8).

Of 426 water samples collected, 120 were collected from outbreak areas and 306 from non-outbreak areas. Although there was not any statistically significant difference, most of the outbreak water samples demonstrated perceptibly higher alkalinity (8.50 $\pm$ 0.25 pH) and salinity levels (0.2 to 1.9 ppt) as compared to those of non-outbreak samples (7.75 $\pm$ 0.2 pH and $\leq$0.4 ppt salinity), and it is worth mentioning that high alkalinity and salinity are conducive for growth and persistence of *V. cholerae* (9).

Most of the outbreak samples (70%) were found to be contaminated with coliform bacteria ranging between 10–75 cfu/mL, whereas only 32% of non-outbreak samples were found to be contaminated with coliform organisms (ranging between 2–25 cfu/mL). It is amply clear thereby that irrespective of any period, coliform contamination reaches a distinctly higher (relative risk [RR] = 2.18; 1.59 < RR < 2.99) threshold prior to any impending outbreak in an outbreak focus.

Altogether 21.6% (92 out of 426) samples were found to be harboring *V. cholerae* along with other coliforms not relevant to the present communication. Among them, 23% of outbreak samples and 21% of non-outbreak samples were found to be positive for *V. cholerae*, with no significant difference between these two groups (Table 1). Of the 92 isolates, 36 were *V. cholerae* O1. However, the isolation rate of *V. cholerae* O1 was significantly higher (RR, 3.18; 1.71 < RR < 5.94) in outbreak samples (16.7%) than in non-outbreak samples (5.2%), which may be attributable to a higher likelihood of fecal or sewage contamination (Table 1). As it is known that the persistence of *V. cholerae* non-O1/non-O139 (56/92, 61%) is common in aquatic environments, the presence of *V. cholerae* O1 indicates either human encroachment (fecal contamination) or environmental factors (due to natural contamination) acting as a carrier, especially in cholera endemic areas (10). Serological analysis revealed that out of 36 *V. cholerae* O1 isolates, 30 were Ogawa and the remaining 6 were Inaba. Among 36 *V. cholerae* O1 isolates, 10 (9 from outbreak isolates) were harboring *ctxB*. A predominance of *ctxB* El Tor was noted (8/10) in comparison to *ctxB* Classical, which highlights the trend of development of new hybrids that has been reported elsewhere (8).

The exceptionally high prevalence of toxic *V. cholerae* O1 in outbreak samples amply signifies the role of potable water as a vehicle for cholera transmission. The higher rate of isolation of the etiological agent is attributed to the selection of point of use as well as stored water, wherefrom behavioral practices can also be predicted (11).

The overall outcome of this study underlines a distinguishing feature among the *V. cholerae* isolates between outbreak and non-outbreak samples. Retention of toxicity in outbreak isolates of *V. cholerae* O1 implicates their pathogenic potential. On the contrary, the isolates of non-outbreak samples were mostly representative of aquatic environmental fauna (*V. cholerae* non-O1/non-O139 and non-toxic *V. cholerae* O1) and a few potentially pathogenic organisms. Nevertheless, the lesser but persistent presence of pathogenic *V. cholerae* in non-outbreak areas also has the potential to blossom into a pathogenic vibrio load (in a potable water system) when a conducive environment, i.e., natural calamities or seasonal changes, prompts genetic conversion of non-pathogenic strains into pathogenic strains (10,12), which can transform a previously non-endemic focus into an endemic/epidemic focus.

Thus, screening of accessible water sources in an endemic focus is a direct investment towards the monitoring and evaluation of a pathogenically engineered transmission vehicle, i.e., water.

Conflict of interest None to declare.

REFERENCES

1. Sengupta, P.G., Sircar, B.K., Mondal, S., et al. (1989): An El Tor cholera outbreak in an endemic community of Calcutta. Indi-

an J. Public Health, 33, 21–25.
2. Colwell, R.R., Kaper, J.B. and Joseph, S.W. (1977): *Vibrio cholerae, Vibrio parahaemolyticus* and other vibrios: occurrence and distribution in Chesapeake Bay. Science, 198, 394–396.
3. Chatterjee, S., Ghosh, K., Raychoudhuri, A., et al. (2009): Incidence, virulence factors, and clonality among clinical strains of non-O1, non-O139 *Vibrio cholerae* isolates from hospitalized diarrheal patients in Kolkata, Indian J. Clin. Microbiol., 47, 1087–1095.
4. Islam, M.S., Hasan, M.K., Miah, M.A., et al. (1994): Isolation of *Vibrio cholerae* O139 synonym Bengal from the aquatic environment in Bangladesh: implications for disease transmission. Appl. Environ. Microbiol., 60, 1684–1686.
5. Deen, J.L., vonSeidlein, L., Sur, D., et al. (2008): The high burden of cholera in children: comparison of incidence from endemic areas in Asia and Africa. PLoS Negl. Trop. Dis., 2, e173. doi:10.1371/journal.pntd.0000173.
6. Mahalanabis, D., Lopez, A.L., Sur, D., et al. (2008): A randomized, placebo-controlled trial of the bivalent killed, whole-cell, oral cholera vaccine in adults and children in a cholera endemic area in Kolkata, India. PLoS One, 3, e2323.
7. Nandi, B., Nandy, R.K., Mukhopadhyay, S., et al. (2000): Rapid method for species-specific identification of *Vibrio cholerae* using primers targeted to the gene of outer membrane protein OmpW. J. Clin. Microbiol., 38, 4145–4151.
8. Morita, M., Ohnishi, M., Bhuiyan, N.A., et al. (2008): Development and validation of a mismatch amplification mutation assay PCR to distinguish between the cholera toxin B subunit of classical and El Tor biotypes of *Vibrio cholerae* O1. Microbiol. Immunol., 52, 314–317.
9. Miller, C.J., Drasar, B.S. and Feachem, R.G. (1982): Cholera and estuarine salinity in Calcutta and London. Lancet, May 29, 1216–1218.
10. Meibom, K.L., Blokesch, M., Dolganov, N.A., et al. (2005): Chitin induces natural competence in *Vibrio cholerae*. Science, 310, 1824–1827.
11. Tambekar, D.H., Gulhane, S.R., Jaisingkar, R.S., et al. (2008): Household water management: a systematic study of bacteriological contamination between source and point-of-use. Am. Euras. J. Agric. Environ. Sci., 3, 241–246.
12. Palit, A. and Batabyal, P. (2010): Toxigenic *Vibrio cholerae* from environmental sources associated with the cholera outbreak after 'AILA' cyclone in West Bengal, India. Lett. Appl. Microbiol., 51, 241–243.