J. Adv. Sci. Edu. Res. **2020: 1: 12-21,** ISSN: 2583-0155 (ONLINE)
Published: 25.12.2020 (http://jaser.rkmvccrahara.org/)

ENVIRONMENTAL PARADIGM OF TOXIC *V. cholerae* IN GANGETIC DELTA OF INDIA

Subham Mookerjee [1,2]*, Suvajit Saha[2] , Anup Palit[2] , Madhumanti Halder[2], Prasenjit Batabyal[2]

[1] Department of Zoology
Ramakrishna Mission Vivekananda Centenary College, Rahara, Kolkata

&

[2] Department of Bacteriology
National Institute of Cholera and Enteric Diseases, Beliaghata, Kolkata

Abstract : Cholera is a recurring problem despite the vaccination. We have attempted to understand the seasonal dynamics of *Vibrio cholerae* in the Gangetic delta and the role of environmental attributes in toxin acquisition in them. 145 (22.5%) *V. cholerae* were isolated comprising of 13.1% *V. cholerae* O1 from four sites off river Ganges. Prevalence of *V. cholerae* O1 at 0.1 - 4.0 PSU salinity indicates higher transmission potential at low saline settings although being present at all sites. We have demonstrated that 32±2°C water temperatures, 800±150 NTU turbidity and 3.0 ±1.0 PSU salinity are the inducing factors facilitating toxicity acquisition in estuarine *V. cholerae* as they migrates inland by tidal pressure in the riverine system. Simultaneously in an in vitro experiment, we have identified that water temperature, salinity and turbidity, at their optimum ranges induces toxicity acquisition amongst environmental *V. cholerae* from the aquatic system, harbouring different virulence associated genes, resulting in Bengal diarrhoea menace. The application of a modified technique *in vitro,* for the determination of the optimal physicochemical conditions, responsible for the horizontal gene transfer in the environmental *V. cholerae* isolates and their ability to cause Cholera has also been elucidated.

Keywords: *Vibrio cholerae* O1, Toxicity, Ganga River, Turbidity, Salinity, Horizontal Gene Transfer.

Introduction

Cholera epidemics usually occur with seasonal regularity in the Gangetic delta of India (Colwell 1996). Epidemics generally occur twice a year (Alam et al. 2011) with the highest number of cases in the month of July-October. A resurgence of cholera cases associated with significant morbidity and mortality has been perceptible among adjoining community of the Gangetic delta of West Bengal, despite of a post cholera vaccination phase, when the number of cholera vis-s-vis diarrheal cases are expected to be very low (Bhattacharya et al. 2013, Batabyal et al. 2013, Sur 2011). Acute gastrointestinal diseases are caused by a variety of micro-organisms including bacteria, viruses and protozoan. 15–20% of community diarrheal disease in developing countries is attributed to unsafe drinking water. Recent studies indicating higher public health concerns due to water borne diseases, with higher incidence rate of diarrheal disease in both developed and developing countries (Mookerjee et al. 2014a, Palit et al. 2012). Vibrios are one of the most common etiological agent of diarrheal disease including cholera; gastrointestinal infections, septicaemia etc. (Saha et al. 2019, Mookerjee et al. 2015).

Vibrio cholerae is the causative agent of cholera (Batabyal et al. 2012). Cholera is an important cause of morbidity and mortality in many developing countries in Asia, Africa, and Latin America due to lack of safe water supply and poor hygienic practices (Colwell 1996). Most of the cholera pandemics started in the Ganges–Brahmaputra delta, which is considered to be the homeland for cholera since ancient times (Batabyal et al. 2012, Drasar & Forest 1996). In tropical countries like India, with low-lying coastal belts, there is a need for establishing evidential links between hydrology and the ecology of diarrheal agents (Wolanski et al. 2004) but reports on the prevalence and densities of *Vibrio* and its implication alongside the inland riverine environment were very sparse.

**For Correspondence.*
(email: subham.mookerjee@gmail.com)

Cholera epidemics can be related to seasonal cycle and physico-chemical properties of water (Singleton et al. 1982). Catastrophic environment has been singled out as an environmental inducing factor for transformation of non-toxic *V. cholerae* to toxic one (Palit & Batabyal 2010). Studies have been initiated to monitor the prevalence of *V. cholerae* O1 in different aquatic ecosystem.

It has been postulated that aquatic environment harbors virulence associated genes scattered among environmental *Vibrio*s with a lower virulence potential than the epidemic strains. In the aquatic environ, *V. cholerae* genetic elements mediates gene transfer and the mammalian host possibly supports the clustering of critical virulence genes in an amiable combination leading to the evolution of new *V. cholerae* strains with epidemic potential (Faruque & Nair 2002).

However, no such environmental inducing factor has been emphatically singled out, which can be responsible for bacterial toxicity acquisition due to genetic modulation in aquatic environment. Adding to this, role of riverine-estuarine ecosystem on *Vibrio* dynamics has never been targeted in Indian part of Gangetic delta to explore its potentiality in cholera transmission. The present study is thereby a cumulative effort, first of its kind, to effectively address this void of knowledge associated to riverine-estuarine ecosystem and *Vibrio cholerae* dynamics in relation to cholera transmission pattern in the south-eastern part of Indian subcontinent.

Methods

Study area

Water samples were collected from four different preselected stations from river Ganges at a fortnightly interval. The river flows through the densely populated south-eastern part of India, a century old cholera endemic region, before opening into the Bay of Bengal (Fig. 1). Apart from drinking purposes, river water is also used for multiple purposes like navigation, washing, bathing, cleaning utensils etc.

Four sampling stations were:

- 'Namkhana' (20km away from sea mouth, 21.76° N, 88.23° E, site-1),
- 'Kakdwip' (40 km away from sea, 21.88° N, 88.18° E, site-2),
- 'Diamond Harbour' (80km away from sea, 22.20° N, 88.20° E, site-3) and
- 'Howrah' (within the city of 'Kolkata' and 130km away from sea, 22.59° N, 88.31° E, site-4) (fig.-1).

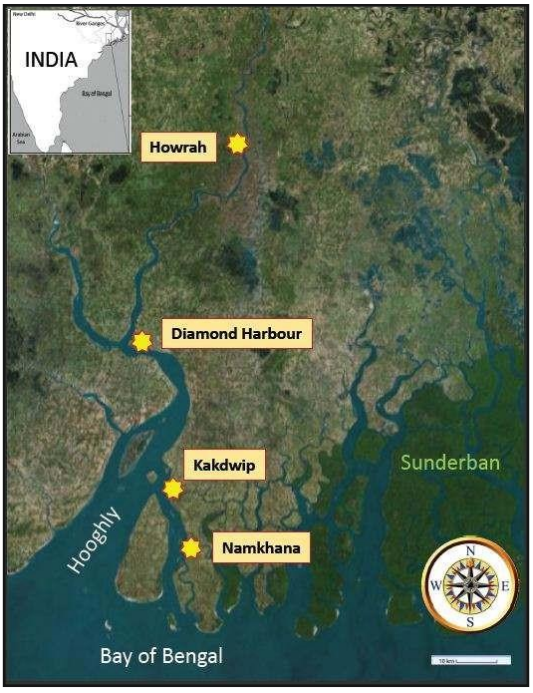


Figure 1. Study area showing 4 Sampling sites

Water sampling and physico-chemical analysis

Water samples were collected in sterile 500 ml. glass bottles from about 50 cm underneath the water surface. At each sampling station 12 - hourly sampling has been conducted. Physico-chemical indices like temperature, pH, turbidity, conductivity and salinity were determined using appropriate probes (Thermo Orion) (Palit & Batabyal 2010).

***Vibrio cholerae* isolation and identification**

Isolation and identification of *V. cholerae* from water samples was undertaken applying modified technique as described elsewhere (Palit & Batabyal 2010). Briefly, 100 ml of water samples was filtered through 0.22µm membrane filter (Millipore Corp., Bedford, MA, USA) and enriched in 10 ml of modified alkaline peptone water (Becton Dickinson, Sparks, MD) (APW prepared using water collected off the same site and passed through 0.22 µm membrane filter) at 37^{O}C for 18 h. Thereafter, 0.5 ml of each of the enriched sample was streaked on TCBS (Thiosulphate citrate bile salt sucrose agar; Becton Dickinson) agar and incubated at 37^{O}C for 18–24 h. Suspected *V. cholerae* isolates were subjected to a simplex PCR technique for identification of *V. cholerae,* which amplifies the 588 bp *OmpW* gene (Palit & Batabyal 2010). *V. cholerae* isolates, confirmed through PCR, were serologically tested (*V. cholerae* O1 Poly, Ogawa and Inaba [BD] antisera). *V.cholerae* O1 isolates were characterized by multiplex PCR for toxin producing genes viz. *ctx* and *tcp* (classical and El Tor) (Keasler & Hall 1993).

In vitro experiment

Here we attempted to grow separately 1 ml of toxic (ctx^{+}, tcp^{+} Kan^{r}) *V. cholerae* O1 and non-toxic *V. cholerae* O1 (ctx^{-}, tcp^{-}, Kan^{r+}) in APW (Merck, Germany) for obtaining the log phase. After 4-6 hrs of incubation at 37^{o}C, 2 ml of each culture was mixed in a single tube, containing 10 ml of defined artificial seawater medium (DASW, pH 7.4) (Meibom et al. 2005). The experiment has been modified in a way to replicate the same 10 times (varying the salinity range from 1 to 10 ppt) and again each level of salinity has been replicated 10 times with different turbidity levels (from 100 to 1000 NTU). Appropriate positive as well as negative control has also been included in the experimental set.

After static 24 hrs incubation at 37^{o}C, all the experimental sets were inoculated separately in a kanamycin containing medium. For molecular identification, each colony was subjected to PCR based identification targeting *V. cholerae* specific o*mpW* and *ctx-tcp* genes (Keasler & Hall 1993, Palit & Batabyal 2010).

Haemolysis Assay

V. cholerae isolates that were PCR positive for toxin gene were tested for haemolysis activity on Sheep Blood agar plates (HI-MEDIA) which showed lysis zone and break-down of RBCs (Halder et al. 2017).

Statistical Analysis

The results have been analysed statistically applying correlation regression methods to compare the physico-chemical constituents of water samples and presence of *V. cholerae* O1 (Table 1). For comparisons of parameters, each and every sample data was considered. For linear regressions and further correlations, probability values < 0.05 were considered significant. The analysis was done by using Epi Info (Ver 3.5.1. USA).

Results and Discussion

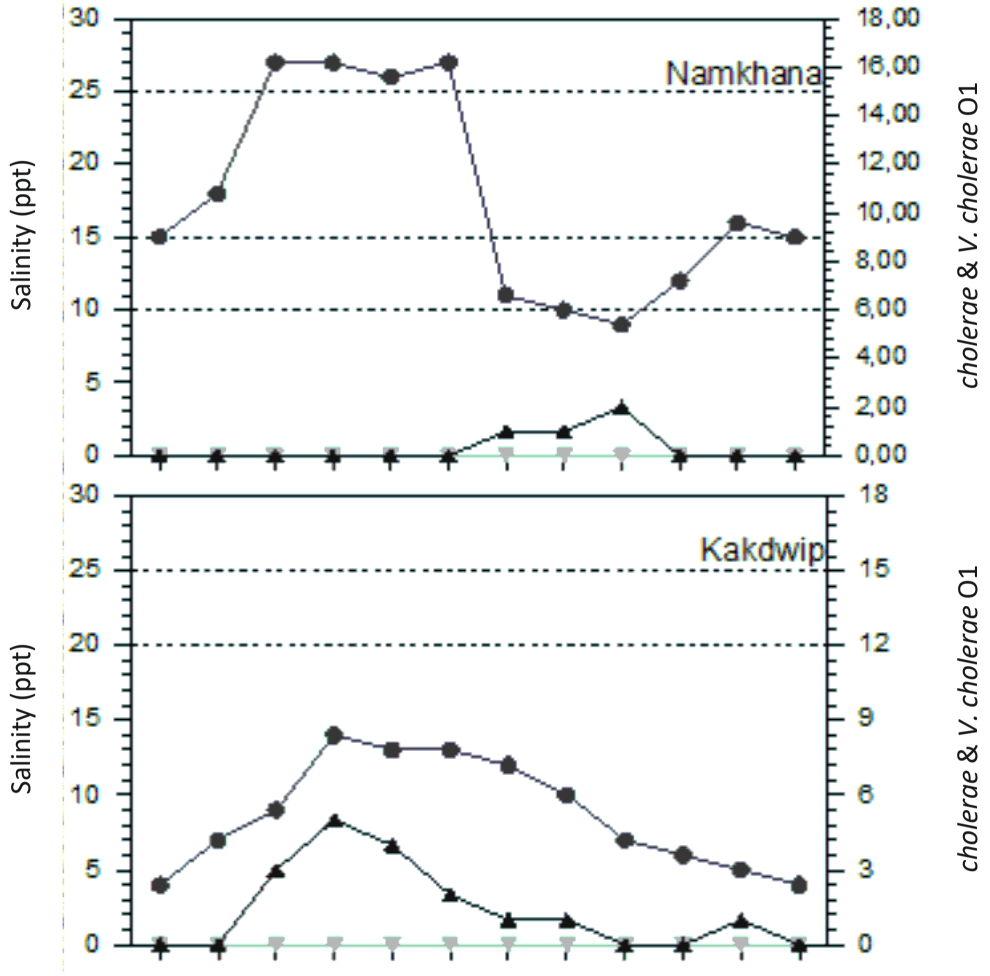
Physico-chemical Analysis

600 water samples have been collected from the 4 (four) riverine-estuarine sites. Water temperature varied between 10^{o}C to 37^{o}C between all the sampling sites, with very high temperatures were recorded in the summer and monsoon season and low temperatures were recorded in the winter. pH ranged from 7.6- 8.2 at all the study sites, showing a general alkaline nature of the river water, at all the sampling sites, throughout the year. Salinity gradient of riverine environment varied between 27.5 PSU (at site-1) to 0.1 PSU (at site-4) gradually diminishing with the increasing distance from the sea mouth (Table 1). Distinct oscillation in salinity level has been detected in response to tidal variation at site 3 (0.5 – 4 PSU) and site 2 (3.4 – 13.5 PSU), where high tide brings in higher amount of marine saline water thus increasing the salinity. In spite of having two distinct salinity zone at site-1 and 4 (24.8-27.5 PSU and 0.1- 0.2 PSU respectively), seasonal variations and tide has no significant influence on salinity at these sites. Turbidity ranged between 12-980 NTU at different sites, with more turbid waters at site-3 and 4 all-round the year (Table 1). However, a uniformly elevated turbidity level has been noted at all the sites after heavy downpours or natural calamities.

***V. cholerae* Analysis**

Altogether, 145 *V. cholerae* have been isolated, out of which 19 isolates (3.2% of total sample) were *V. cholerae* O1 (Table 1). Most of the *V. cholerae* isolates have been isolated from site-3 and 4 and much lower persistence of *V. cholerae* isolates has been observed at site-1 and 2. Thus a comparatively low salinity (4 - 0.1 PSU) coupled with higher turbidity (23-980 NTU) seems to be the favorable niche for *V. cholerae* persistence and proliferation. Increase in preponderance of *V. cholerae* towards inland (low salinity), than that of the sea mouth (comparatively higher salinity region), not only specifies the adaptability of these *V. cholerae* in a low saline environment by osmoregulation but also elucidates their capability to survive in potable water sources. Among the study sites, highest isolation rate of *V. cholerae* O1 has been detected at Howrah site (13 of 150; 8%), followed by Diamond Harbour site (6 of 150; 4%), which indicates the higher prevalence of *V. cholerae* O1 in the inland, increasing manifold the chances of disease transmission to the community coming in contact with the inland river water and also implies the role of riverine– estuarine ecosystem in cholera transmission (Mookerjee et al. 2014).

Distinct seasonality has been observed both at Howrah as well as Diamond Harbour. Most of the *V. cholerae* O1 at Howrah were isolated in summer and monsoon period, while at Diamond Harbour, all *V. cholerae* O1 could be isolated in summer months only (fig. 2). Statistically significant correlation has been observed between turbidity and *V. cholerae* O1 disposition ($r=0.87$, $p>0.001$), salinity and *V. cholerae* O1 disposition ($r=0.79$, $p>0.01$) at Diamond Harbour than that of Howrah site.



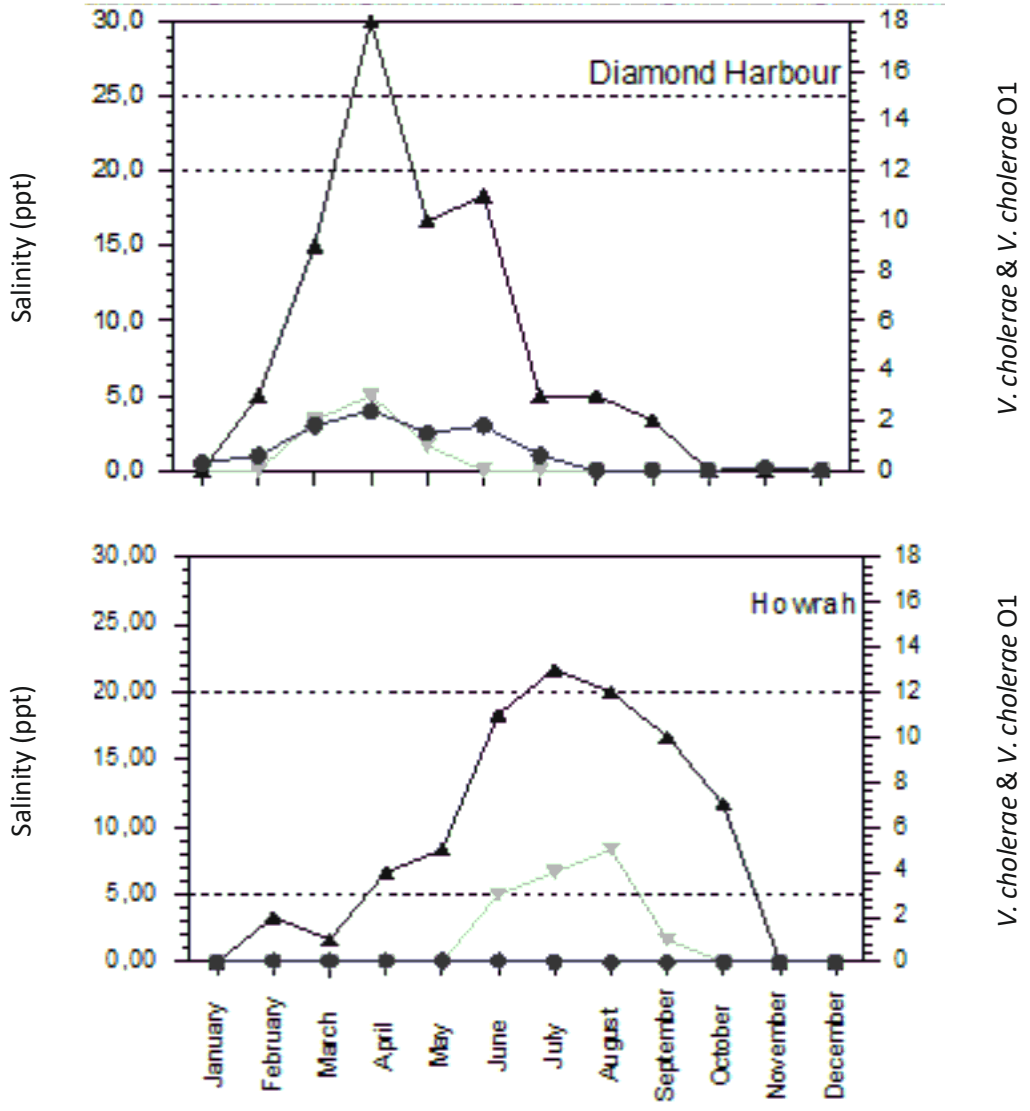


Figure 2. Site wise seasonal variation of salinity (●), *V. cholerae* (▲), *V. cholerae* O1 (△)

Toxic *V. cholerae*

Toxicity determination in environmental *V. cholerae* revealed that among 19 *V. cholerae* O1, 10 isolates harbor *ctx* and *tcp* gene, whereas 2 isolates were only with *tcp* gene (Table 1). Among toxic *V. cholerae* O1, 7 isolates have been isolated from site-4 and 5 isolates from site-3 without any significant statistical difference of disposition between them (fig 2). At Diamond Harbour site, disposition of toxic *V. cholerae* O1 was mostly observed in summer and remained associated with elevated water temperature (32 ±2°C), higher turbulence and increased turbidity (800±150NTU); whereas predominance of toxic *V. cholerae* O1 at Howrah has mostly been observed during monsoon period with higher turbidity (560-980 NTU) in a swollen river receiving heavy rainfall and run off organic and inorganic debris from the surrounding areas of either sides of the river.

Thereby the dynamics of *V. cholerae* O1 in the river Ganges suggests a combination of parallel existence and effect of two different pools of *V. cholerae* O1. One pool of *V. cholerae* O1 is being swept inland through riverine gateway via tidal and wind pressure from Diamond Harbour site to Howrah. Another pool of *V. cholerae* O1

is simultaneously swept into the river in monsoon through the untreated sewage disposal and flood water from adjoining metropolis carrying in, organic debris inclusive of bacterial community.

On the other hand, presence of toxic *V. cholerae* O1 at Diamond Harbour site during summer months is a natural phenomenon without involvement of any anthropogenic inputs, as evident from the negligible fecal coliform level (Batabyal et al. 2014a). It can further be hypothesized that, *V. cholerae* (in non-toxic form), from high saline zone, migrates into an inland riverine ecosystem in low saline region, in between which they can acquire toxicity from the virulence gene pool already present in the environment under the influence of different environmental factors like higher water temperature (32±2°C), higher turbidity (800±150NTU), mild salinity (3.0 ±1.0 PSU) which either can be created by any catastrophic situation (Palit & Batabyal 2010), in presence of chitin (Saha et al. 2020, Batabyal et al. 2014b) or can be traced at very specific aquatic habitat like the existent one at inland riverine environments.

The summer season at inland Gangetic riverine system can thus be considered as a "buildup season" when it receives nontoxic *V. cholerae* from its downstream (fig. 2) which acquires toxicity enroute inland aquatic environment. A cluster of abiotic attributes viz. temperature, turbidity and salinity in unison can acts as convenient "bio-environmental stimulants" triggering this toxin gene acquisition to induce genetic modification and can enhance toxic *V. cholerae* load in the aquatic environment. Simultaneously, the highest load of toxigenic *V. cholerae* (fig. 2) in the monsoon season in the riverine ecosystem is attributed to the genetically modified downstream *V. cholerae* population and *Vibrio* load of the flood water from adjoining metropolis carrying into the river organic debris inclusive of bacterial community. Therefore, in our attempt to address the interplay of environment and Gangetic riverine-estuarine ecosystem, a distinct resemblance has been observed between the environmental preponderance of toxic *V. cholerae* O1 and seasonal cholera incidence. Our present observation of seasonal abundance of toxic *V. cholerae* O1 at Diamond Harbour in summer followed by Howrah or Kolkata (two densely populated metropolis on either side of the river) during rainy season magnificently tallies with the established cholera peaks (WHO 2010).

V. cholerae & physico-chemical variants

During the present study, it has been observed that the abundance of riverine-estuarine *V. cholerae* is reliant on the physico-chemical properties of the aquatic environment. Statistical analysis revealed that temperature, turbidity and salinity have a greater impact on bacterial abundance including *Vibrio spp*. While pH level showed an insignificant correlation with *V. cholerae* abundance ($P>0.118$; $n=145$), water temperature significantly have a close-fitting correlation with *V. cholerae* O1 ($P<0.0008$; $n=19$) (Table-2). Higher preponderance of *V. cholerae* has always been noted in mid and low saline regions with varied salinity between 7.5 to <0.1 PSU. Therefore, a negative correlation has been observed between salinity and *V. cholerae* pool. Turbidity content also depicted a significant correlation with distribution of *V. cholerae* pool ($p<0.001$; $n=145$) (Table-2). *V. cholerae* isolates also showed a marked seasonality with a distinct steady increase from summer (March-June) to monsoon (July- August) with a steep ascent in the post-monsoon (September- October) season when the physico-chemical properties are optimum. An abrupt decline in the isolation rate of *V. cholerae* isolates was observed in the winter season (November- February) due to unfavorable conditions (Halder et al. 2017). Similar finding has been reported in the waters of the Sunderban Mangrove by Batabyal et al. (2016).

The field observation that specific levels of salinity and turbidity are possible environmental regulators for acquisition of toxin genes in *V. cholerae* prompted us to design and apply a modified technique with the DASW to identify optimal conditions for the same, so that our field observations could be evidentially validated *in vitro*.

In vitro experiment

From the modified *in vitro* analysis we observed that, kanamycin resistant toxic *V. cholerae* O1 could only be isolated from those experimental sets where turbidity level of ≥600 NTU and salinity range of 2-5 PSU (Table-

3) prevails. From this experiment, we infer that a certain optimal level of turbidity and salinity enhance toxicity acquisition via genetic exchange among *V. cholerae* O1 isolates which substantiates our earlier field observations.

Haemolytic Assay

The *Vibrio cholerae* O1 isolates with the toxin genes when inoculated onto the Sheep Blood Agar plates showed distinct haemolysis activity breaking the haemoglobin molecules in the plate producing clear lysis zones. This signifies that the toxin genes that are acquired by the *V. cholerae* isolates are functional and have the potential to induce disease given favourable conditions and therefore increase the risk of pathogenic transmission and occurrence of disease in human subjects.

Conclusion

The dynamics of *V. cholerae* O1 revealed from the present study explains an unknown environmental phenomenon in the Gangetic riverine system where non-toxic *V. cholerae* O1 migrates upstream, to a mild salinity (2-5 PSU) zone in a flowing water course, from their high saline brackish origin at higher water temperature (≥30°C). These environmental conditions coupled with increased turbidity (≥600 NTU) induce the genetic modification among riverine *V. cholerae* O1 community producing the toxic progenies, which is also seen in the in-vitro experiment. The toxic *V. cholerae* O1 are carried inland by the tidal flow in the riverine system and cause cholera in the community coming in contact with the riverine water. We demonstrate for the first time the unique influence of eco-hydrological oscillations in the entire lower Gangetic basin in relation to seasonal variation on the natural transformation of toxic *V. cholerae* O1 in Gangetic riverine-estuarine ecosystem. This strong impact of riverine-estuarine ecology on *V. cholerae* dynamics and its acquisition of toxin genes can be associated with high incidence of cholera along the Gangetic delta of West Bengal, India.

Thus we conclude that the riverine environment and attributes from its adjoining inputs induces acquisition of toxin genes in *V. cholerae* in its conversion into a potential pathogenic pool and naturally confers an increased evolutionary fitness. Bacterial diversification is reportedly driven by a high rate of horizontal transfer, which introduces novel genes, confers beneficial phenotypic capabilities and permits the rapid exploitation of the environment.

References

- Alam, M., Islam, A., Bhuiyan, N., Rahim, N., Hossain, A., Khan, G. Y., Ahmed, D., Watanabe, H., Izumiya, H., Faruque, A. S. G., Akanda, A. S., Islam, S., Sack, R. B., Huq, A., Colwell, R. R. and Cravioto, A. 2011. Clonal transmission, dual peak, and off-season cholera in Bangladesh. Infect. Ecol. Epidemiol., 1(1): 7273.
- Batabyal, P., Einsporn, M.H., Mookerjee, S., Palit, A., Neogi, S.B., Nair, G. B., and Lara, R. J. 2014. Influence of hydrologic and anthropogenic factors on the abundance variability of enteropathogens in the Ganges estuary, a cholera endemic region. Sci. Tot. Environ., 472: 154-161.
- Batabyal, P., Mookerjee, S. and Palit, A. 2012. Occurrence of toxigenic *Vibrio cholerae* in accessible water sources of cholera endemic foci in India. Jap. J. Infect. Dis., 65: 358-360.
- Batabyal, P., Mookerjee, S., Einsporn, M.H., Lara, R.J. and Palit, A. 2014. High prevalence of toxin producing enteropathogenic Vibrios among estuarine crab in Ganges delta of West Bengal, India. Infect. Gen. Evol., 26: 359.
- Batabyal, P., Mookerjee, S., Einsporn, M.H., Lara, R.J., and Palit, A. 2016. Environmental drivers on seasonal abundance of riverine-estuarine *V. cholerae* in the Indian Sundarban mangrove. Ecol. Ind., 69:59-65.
- Batabyal, P., Mookerjee, S., Sur, D. and Palit, A. 2013. Diarrheogenic Escherechia coli in potable water sources of West Bengal, India. Acta. Trop., 127(3): 153-157.
- Bhattacharya, S. K., Sur, D., Ali, M., Kanungo, S., You, Y. A., Manna, B., Sah, B., Niyogi, S. K., Park, J. K., Sarkar, B., Puri, M. K., Kim, D. R., Deen, J. L., Holmgren, J., Carbis, R., Dhingra, M. S., Donner, A., Nair, G. B., Lopez, A. L., Wierzba, T. F. and Clemens, J. D. 2013. 5 year efficacy of a bivalent killed whole-cell oral cholera vaccine in

Kolkata, India: a cluster-randomised, double-blind, placebo-controlled trial. Lancet. Infect. Dis. 13(12):1050-1056.

- Colwell, R. R. 1996. Global climate and infectious disease: The cholera paradigm. Science. 274: 2025-2032.
- Drasar, B.S., Forrest, B.D. 1996. Cholera and the ecology of Vibrio cholerae. Springer Science & Business Media.
- Faruque, S. M. and Nair, G. B. 2002. Molecular ecology of toxigenic *V. cholerae*. Microbial. Immunol., 46: 59-66.
- Halder, M., Mookerjee, S., Batabyal, P. and Palit, A. 2017. Environmental *Vibrio cholerae* non O1/ non O139 from the Gangetic delta: a diarrheal disease purview. Int. J. Env. Health. Res,. 1:11.
- Keasler, S. P. and Hall, R. H. 1993. Detecting and biotyping *Vibrio cholerae* O1 with multiplex polymerase chain reaction. Lancet., 341: 1661.
- Meibom, K.L., Blokesch, M., Dolganov, N.A., Wu, C.Y. and Schoolnik, G.K. 2005. Chitin induces natural competence in Vibrio cholerae. Science., 310(5755): 1824-1827.
- Mookerjee, S., Batabyal, P., Halder, M. and Palit, A. 2014. Specificity of coliphages in evaluating marker efficacy: A new insight for water quality indicators. J. Vir. Met., 208: 115–118.
- Mookerjee, S., Batabyal, P., Sarkar, M. H. and Palit, A. 2015. Seasonal prevalence of enteropathogenic Vibrio and their phages in riverine estuarine ecosystem of South Bengal. Plos. One., 10(9), e0137338.
- Mookerjee, S., Jaiswal, A., Batabyal, P., Einsporn, M. H., Lara, R. J., Sarkar, B., Neogi, S. B., Palit, A. 2014. Seasonal dynamics of Vibrio cholerae and its phages in riverine ecosystem of Gangetic West Bengal: Cholera paradigm. Environ. Monit. Assess., 186(10): 6241-6250.
- Ochman, H. 1998. Molecular archaeology of *E. coli* genome. Proc. Natl. Acad. Sci. USA 95., 9413-9417.
- 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., 5: 241-243.
- Palit, A., Batabyal, P., Kanungo, S. and Sur, D. 2012. In-house contamination of potable water in urban slum of Kolkata, India: a possible transmission route of diarrhea. Water Sci. Technol., 66(2): 299-303.
- Saha, S., Halder, M., Mookerjee, S. and Palit, A. 2020. Preponderance of Multidrug-resistant, Toxigenic, and Thermotolerant Enteropathogenic Bacteria in Raw and Cooked Seafood of Indo-Gangetic Basin and Associated Health Risks. J. Aqua. Food Prod. Technol, 29(9): 838-849.
- Saha, S., Halder, M., Mookerjee, S. and Palit, A. 2019. Seasonal influence, enteropathogenic microbial load and diarrhoeal enigma in the Gangetic Delta, India: Present scenario and health implications. J. Infect. Public. Health., 12(4): 540-548.
- Singleton, F.L., Attwell, R., Jangi, S. and Colwell, R.R. 1982. Effects of temperature and salinity on *Vibrio cholerae* growth. Appl. Environ. Microbiol., 44(5): 1047-1058.
- Sur, D., Kanungo, S., Sah, B., Manna, B., Ali, M., Paisley, A. M., Niyogi, S. K., Park, J. K., Sarkar, B., Puri, M. K., Kim, D. R., Deen, J. L., Holmgren, J., Carbis, R., Rao, R., Van, N. T., Han, S. H., Attridge, S., Donner, A., Ganguly, N. K., Bhattacharya, S. K., Nair, G. B., Clemens, J. D. and Lopez, A. L. 2011. Efficacy of a low-cost, inactivated whole-cell oral cholera vaccine: results from 3 years of follow-up of a randomized, controlled trial. PLoS. Neglect. Trop. Dis., 5(10), e1289.
- Technical Report - World Health Organization, www.who.int/kobe_centre/publications/kolkata_report2011.pdf (retrieved 08-01-2014).
- Wolanski, E., Boorman, L. A., Chícharo, L., Langlois-Saliou, E., Lara, R., Plater, A. J., Uncles, R. J. and Zalewski, M. 2004. Ecohydrology as a new tool for sustainable management of estuaries and coastal waters. Wetlands. Ecol. Management., 12(4) : 235-276.

Table1- Site wise variation of Salinity and Turbidity and microbiological determinants

Study site	**Salinity (PSU)**	**Turbidity (NTU)**	**No. of *V. cholerae* isolated**	**No. of *V. cholerae* O1 isolated**	**No. of toxic *V. cholerae* O1 isolated**
Site -1 (Namkhana)	24.8-27.5	12-150	4	-	-
Site- 2 (Kakdwip)	3.4-13.5	50-280	26	-	-
Site -3 (Diamond Harbour)	0.4-4.7	45-980	50	6	3*+2**
Site -4 (Kolkata)	0.1-0.2	23-475	65	13	7*

**V. cholerae* O1 isolates possessing *ctx* and *tcp* gene

** *V. cholerae* O1 isolates possessing only *tcp* gene

Table 2- Statistical correlation between physico-chemical and microbiological determinants

	pH	Salinity	Turbidity	VC	VCO1	TVCO1
Temperature	**	*	*	*	**	**
pH		*	NS	NS	*	*
Salinity			*	*(-)	*(-)	*(-)
Turbidity				**	**	**
VC					*	*
VCO1						**

****** indicate ($P < 0.001$) highly signi□cant correlation, ***** indicate ($p < 0.05$) signi□cant correlation, **NS** indicate ($p > 0.05$) not signi□cant, **VC**-*V. cholerae*, **VCO1**- *V. cholerae* O1, **TVCO1**-Toxic *V. cholerae* O1

Table-3 Effect of salinity and turbidity variation on genetic transfer in *V. cholerae* O1 in the In-vitro experiment.

Turbidity / Salinity	100 NTU	200 NTU	300 NTU	400 NTU	500 NTU	600 NTU	700 NTU	800 NTU	900 NTU	1000 NTU
1 ppt	–	–	–	–	–	–	–	–	–	–
2 ppt	–	–	–	–	–	+	+	–	+	+
3 ppt	–	–	–	–	–	+	+	+	+	+
4 ppt	–	–	–	–	–	+	+	+	+	+
5 ppt	–	–	–	–	–	+	–	–	+	+
6 ppt	–	–	–	–	–	–	–	–	–	+
7 ppt	–	–	–	–	–	–	–	–	–	–
8 ppt	–	–	–	–	–	–	–	–	–	–
9 ppt	–	–	–	–	–	–	–	–	–	–
10 ppt	–	–	–	–	–	–	–	–	–	–

-indicate no isolation of kanamycin resistant toxic *V. cholerae* O1

\+ indicate isolation of kanamycin resistant toxic *V. cholerae* O1