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Letter To the Editor

High prevalence of toxin producing enteropathogenic *Vibrios* among estuarine crab in Ganges delta of West Bengal, India

Chitin influences the survival of *Vibrio* spp. in aquatic environment by providing an abundant source of carbon, nitrogen and protection from environmental challenges (Kirn et al., 2005). Huq et al. (1986) have demonstrated a possible ecological correlation between crustaceans and *Vibrio* abundance. However, active role of such common edible seafood viz. crabs etc. of aquatic habitat (freshwater/estuarine/marine) as prospective "reservoir" (because of being proven niche of *Vibrio cholerae* in such environment, Blokesch and Schoolnik, 2007) in cholera and non-cholera diarrhoeal transmission and their possible implications among coastal population of deltaic West Bengal remains unexplored. A few earlier studies have demonstrated the attachment capacity of *V. cholerae* with chitinous substrate (Kaneko and Colwell, 1975; Tarsi and Pruzzo, 1999). This raise queries regarding the extent to which chitin acts in toxicity acquisition under natural environmental condition either being attached with a host specific chitinous substrate or in a free floating condition.

Accordingly transmission feasibility of enteropathogenic *Vibrios* through common edible estuarine crabs was explored in river-estuarine setting of Ganges delta of West Bengal.

Water samples and common edible live crabs (*Scylla serrata*) were collected from riverine-estuarine sources of West Bengal, India (Fig. 1). Fifty crabs of river-estuary sources, 50 estuarine water samples and 10 sediment samples were collected for determining the Cultivable Vibrio Count (CVC) as well as enteropathogenic *Vibrio* isolation.

Exoskeleton, gill and gut of collected crab samples were aseptically weighed and homogenised. One gram of homogenised body part was mixed with 10 mL of PBS solution and diluted to 1:10,000 times. 200 μL of each diluted sample was spread on Thiosulphate Citrate Bile salts Sucrose agar (TCBS agar; BD/DIFCO; Sparks, MD, USA) for Cultivable Vibrio Count (CVC). Remaining body parts were enriched in Alkaline Peptone Water (APW, BD/DIFCO) and cultured on TCBS agar with an overnight incubation at 37 °C.

On the other hand, water and sediment samples were processed for detection of CVC (Batabyal et al., 2014) as well as isolation and identification of enteropathogenic *Vibrios* (*V. cholerae* and *Vibrio parahaemolyticus*) following published protocols (Nhung et al., 2007). Molecular identification were conducted following conventional protocols targeting *ctx* and *tcp* gene for *V. cholerae* (Keasler and Hall, 1993) and *tdh* and *trh* gene for *V. parahaemolyticus* (Tada et al., 1992).

Cultivable vibrio count (CVC), when enumerated, was highest from external chitinaceous shell (mean 7×10^7 CFU/gm) of crabs, followed by gut (mean 4×10^6 CFU/gm) and gills (mean 4×10^6 CFU/gm) respectively, reflecting the undisputed affinity of *Vibrio* sp. towards chitinous substrates as an attachment enhancing factor. In riverine-estuarine water samples CVC ranged between 1 and 880 CFU/mL. Prevalence of cultivable *Vibrios* varied according to the seasonal fluctuation (Fig. 2). Increased water temperature influences the higher predominance of *Vibrio* organisms in summer months (10–880 CFU/mL) followed by a fairly high distribution in monsoon months (7–175 CFU/mL) and then a sudden fall along with the onset of winter (1–100 CFU/mL). On the other hand, higher water temperature indicate lower preponderance of enteropathogenic *Vibrios* among sediments samples in summer and monsoon and a gradual increase in CVC during winter (Fig. 2). This phenomenon might be explained by higher affinity of aquatic vibrio pool to go into attachment with benthic planktonic substrates in winter months by formation of biofilms. While summer and monsoon CVC among sediment samples ranged between 200 and 600 CFU/gm; it substantially increases in winter months ranging between 800 and 2000 CFU/gm. Therefore, higher prevalence of free floating *Vibrios* in water in conducible summer and monsoon months is amply evident in the above observation.

40% (20/50) estuarine water samples were detected to harbour enteropathogenic *Vibrio* spp. with identification of *V. cholerae* non-O1/O139 (16%), *V. parahaemolyticus* (38%), based on their molecular characteristics as stated earlier, establishing a lesser persistence of toxin producing *V. cholerae* in free flowing saline ($\geqslant$15 ppt) water than that of *V. parahaemolyticus*. Only one water sample has been detected to harbour toxin producing *V. parahaemolyticus* possessing *tdh* as well as *trh* gene.

From sediment sample analysis, it was revealed that, while 60% samples harboured *V. parahaemolyticus,* only 30% of samples were positive for *V. cholerae* non-O1/O139. Rare but presence of toxin producing *V. parahaemolyticus (tdh* +ve) has been encountered from one estuarine sediment sample also.

As high as 52% (26/50) of collected crabs were detected harbouring *V. cholerae*, of which 20% (10/50) were *V. cholerae* O1 Ogawa (Table 1), a distinctly different and inverse observation compared to two other sources (water and sediment). All those *V. cholerae* O1 were isolated from crab chitinous shell surfaces demonstrating their preferable adherence with shell surfaces than that of a free floating state in the estuarine environment (viz., water, sediment, etc.). Out of 10 *V. cholerae* O1, 4 isolates were positive for *ctx* and *tcp* and 2 had only *tcp* gene (Table 1). These entire toxins producing *V. cholerae* O1 were of El Tor biotype.

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Fig. 1. Study area wherefrom the crab specimens, water and sediment samples have been collected. ▲ Sites, from where crabs and water samples have been taken.

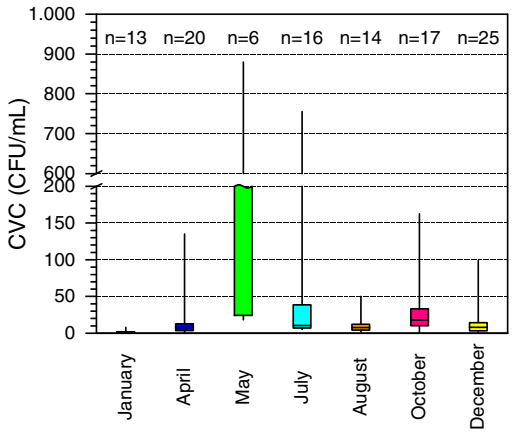

Fig. 2. Seasonal fluctuation of cultivable Vibrio in riverine environment.

Isolation of toxin producing *V. cholerae* O1 from chitinous shells explains two facts of remarkable importance of chitin, one as a nutrient source (Kirn et al., 2005) and other fact that chitinaceous fauna like edible crabs (in our observation) are active reservoirs and potential transmission vehicle for toxin producing *V. cholerae* from natural aquatic source to community level.

Detection of *V. parahaemolyticus* from crabs has almost been a regular observed phenomenon since 90% (45/50) of the crab samples were found to harbour them. Among them, 30% (15/50) were found to harbour toxin producing *V. parahaemolyticus* containing *tdh* and *trh* genes. In another way, one third of the *V. parahaemolyticus* containing crabs were presumable reservoirs as well as potential capable transmitters of toxin producing *V. parahaemolyticus* inducing gastrointestinal disorders.

Higher isolation of toxin producing *V. cholerae* ($P < 0.0001$) and *V. parahaemolyticus* ($P < 0.0001$) from crab samples (from chitinaceous shell surface) than that of other sources is statistically significant. This also indicated a distinct possibility of chitin induced genetic modification after bacterial attachment. Moreover, very low detection of toxin genes among free floating or sediment colonised *Vibrios* explains the explicit role played by chitinous host as most suitable niche for genetic modification.

Hence, considerably higher isolation of toxin producing *V. cholerae* O1 and *V. parahaemolyticus* from estuarine crabs indicate that crab plays the dual role of both concomitant transmission and transformation of *Vibrios* from non-toxigenic to toxigenic progeny. This environmental phenomenon most possibly and significantly precedes conversion of avirulent *V. cholerae* and *V. parahaemolyticus* into a virulent progeny in aquatic milieu as has been previously reported by Palit and Batabyal, 2010 from coastal estuarine foci of West Bengal.

Therefore, our findings suggest that among multiple hosts in aquatic condition, enteropathogenic *Vibrios* prefer to colonise on crabs (as a chitinous substrate) and possibly acquire their toxicity during the attachment phase under the influence of chitin. Further in-sight studies are required to establish the mechanism of genetic modification that will contribute to the cholera as well as diarrheal epidemiology.

Table 1
Distribution of enteropathogenic *Vibrios* in different components of aquatic ecosystem.

Samples	Total No. of samples analysed	CVC	*V. cholerae*+ve sample (%)	Toxin producing *V. cholerae* +ve sample (%)	*V. parahaemolyticus* +ve sample (%)	Toxin producing *V. parahaemolyticus* +ve sample (%)
Water	50	1–880 CFU/mL	8 (16%)	–	19 (38%)	1 (2%)
Sediment	10	200–2000 CFU/gm	3 (30%)	–	6 (60%)	1 (10%)
Crab	50	$\approx 10^7$ CFU/gm	26 (52%)	6 (12%)	45 (90%)	15 (30%)

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