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Environmental drivers on seasonal abundance of riverine-estuarine *V. cholerae* in the Indian Sundarban mangrove

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ABSTRACT

Gangetic delta is considered as the homeland of cholera, which is thought to be influenced by changes in populations of estuarine *Vibrio cholerae*. We aim to identify the environmental, biotic and abiotic driving forces influencing the *V. cholerae* dynamics in riverine-estuarine environment of southern deltaic Bengal. Cultivable Vibrio count (CVC) ranged between 1 and 10^3 colony forming units (CFU)/mL at a salinity gradient of 1.9–30 practical salinity unit (PSU). Increased water temperatrure during summer influences the higher CVC followed by a sudden fall along with the onset of monsoon upto winter. While summer *V. cholerae* O1 peak (50–100 CFU/mL) can be associated with higher water temperature ($P < 0.05$) and higher turbidity ($P < 0.005$); sharp fall during monsoon (15–45 CFU/mL) is attributed to reduced salinity (25–2.5 PSU). Plankton attached *V. cholerae* O1 varied between 10 and 1000 CFU/mL with a highest peak at winter followed by summer and monsoon. Prevalence of toxigenic *V. cholerae* O1 in low salinity (2–7.5 PSU) during monsoon identifies that high water temperature (>25 °C), higher turbidity (>100 NTU) and lower salinity plays the pivotal role in toxicity acquisition. Present investigation establishes the role of Sundarban mangrove, where *V. cholerae* exist in an avirulent condition. During migration towards low saline inland system, *V. cholerae* pool possibly acquires toxin genes under the influence of environmental factors. Planktonic attachment is possibly a survival strategy at adverse condition, when they do not acquire any toxin gene. Seasonal *V. cholerae* dynamics has been thoroughly established in environmental settings of high saline mangrove and brackish water flowing to inland low saline condition.

1. Introduction

Vibrio cholerae, the causative agent of cholera, although a human pathogen is nearly native to all aquatic environments (Mookerjee et al., 2014). Cholera is endemic in those regions where inadequate sanitary practices and consumption of contaminated water and food are common. It has been estimated that likely 15–20% of community diarrheal disease in developing and developed countries is attributed to unsafe drinking water with recent studies indicating even higher percentages of waterborne diarrheal disease (Palit et al., 2012). Toxigenic strains of *V. cholerae* serogroups O1 and O139 have been identified with cholera epidemics and pandemics (De Magny et al., 2011). *V. cholerae* is part of the autochthonous flora of brackish and estuarine environments (Batabyal et al., 2012). *V. cholerae* can be found as a non-symbiotic 'free-living' organism in the water column and also attached to phytoplankton, zooplankton and other aquatic organisms (Islam et al., 1994; Tamplin et al., 1990). *V. cholerae* can secrete extracellular enzymes like chitinase and mucinase which can aid in the adherence and growth of the bacterium on chitinous or mucilaginous organisms like copepods, shrimps, blue green algae, etc. (Batabyal et al., 2014a; Huq et al., 1983; Nalin et al., 1979).

Over the last 3 decades, the major cholera epidemics, including the first outbreak of *V. cholerae* O139 disease have originated in coastal areas. Currently, the main foci of cholera endemicity include the coasts surrounding the Bay of Bengal, both Bangladesh and the Indian subcontinent which is considered as the homeland of cholera, and coastal Latin America (Lipp et al., 2002; Batabyal et al., 2012) and Africa. In the low lying Gangetic delta of West

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Bengal, the seawater intrusion is a common incident. Again Robert Koch in 1984 suggested Sundarban mangrove as a principal source of cholera outbreaks where the combination of brackish, organic matter rich coastal water and high human population density in upstream areas can provide ideal condition for *V. cholerae* proliferation. Thus small increase in sea-level may have a significant impact on the water regime of the large Sundarban mangrove in the south-eastern part of India and induce *V. cholerae* proliferation.

High temperature, salinity and pH can favour survival of Vibrios in aquatic environments (Valipour, 2015; Mookerjee et al., 2015; Batabyal et al., 2014b; Louis et al., 2003; Mahmud et al., 2008). However longitudinal field-based investigations on the interaction between *V. cholerae* abundance and environmental drivers are limited. Some studies have investigated the dynamics of estuarine and coastal *V. cholerae*, but most of them were conducted in regions where cholera is not endemic and did not systematically observe the habitational and mutagenic transformations of "free-living" and plankton-associated populations of toxigenic O1 and O139 strains from high saline to freshwater zones (Barbieri et al., 1999; Castañeda Chávez et al., 2005; Colwell et al., 1981; Louis et al., 2003). The differential occurrence of "free-living" and attached *V. cholerae* suggests a significant role of plankton in spread of *V. cholerae* into freshwater areas (Neogi et al., 2012).

However, in this cholera endemic zone, apart from a few short term approaches (Lara et al., 2011; Neogi et al., 2012) in Bangladesh part of the Sundarban mangrove, the ecology of toxigenic *V. cholerae* in a different but identical coastal environment such as mangroves, marshes and estuaries have never been targeted. Thus the role of the different environmental factors are needed to be explored to provide a very important knowledge base that can act as a platform for an early warning system to understand human vulnerability to this dreaded disease (De Magny et al., 2008). Moreover the role of seasonality and environmental drivers on planktonic attachment/detachment followed by toxicity acquisition among environmental *V. cholerae* isolates are also required to be explored to add further new dimensions to critically evaluate the role of mangrove and brackish water environment in Bengal cholera menace.

Here we aim to identify the environmental driving forces influencing the *V. cholerae* dynamics in the riverine-estuarine environment of southern deltaic Bengal. Different biotic and abiotic factors have been considered to recognize their role as environmental attributes in origination and transmission of toxigenic *V. cholerae*, from its natural habitat to inland human population.

2. Materials and methods

2.1. Study sites

Sundarban, world's largest delta is formed by the confluence of rivers Ganges, Brahmaputra and Meghna and lies in the south-eastern part of West Bengal, India. Sundarban covers an area of 4262 km^2 in India. Sundarban mangrove ecosystem dominated by mangrove forests and derives its name from the Sundari trees (*Heritiera littoralis*) being spread over 54 islands. The Matla and the Vidyadhari River flows around these islands forming many creeks which are tidally influenced. This area is imprecisely separated into low depth, mudflat and typical riverine region which can only be distinguished during low tide. Anthropogenic intrusion is negligible, as the usage of the riverine water is restricted due to its salinity and minimum accessibility. Except fisheries, tourism and honey collection, other activities have rarely been observed.

Between January 2011 and January 2012, altogether 8 transect cruises have been undertaken, starting from the northern most navigable point to the opening of the river into Bay of Bengal. Altogether, 16 equidistant sampling points were fixed in the riverine-estuarine systems of the Sundarbans on the basis of navigability and the uniqueness of the spot (meeting point of creeks, tributaries, smallest river width, etc.) (Fig. 1).

Sampling was carried out from aforementioned sampling sites, cruising from inland low saline water zone up to the estuary's mouth (50 km away) sailing against tides, till reaching the marine water sector and then returning along the same transect. At each sampling station, three sub-samples of surface water were collected from the middle of the river channel at a depth of 0.5 m from the river water surface (i.e. just below the water surface), with sterilized buckets and pooled together, in a sterile 10 L container. Subsequently, the samples were shaken and divided into 1 L aliquots. After collection, water samples were stored in ice-chilled, lightproof boxes and transported to the central laboratory for further processing and analysis. In this eight sampling expedition across one year, a total number of 143 surface water sample sets were collected.

2.2. Fractionation

In each expedition, 4 fractionations were undertaken from same sampling spots and processed as previously reported elsewhere (Alam et al., 2006; Lara et al., 2011; Neogi et al., 2012). Briefly, 300 L of water was filtered successively through 200 μm, 55 μm and 20 μm mesh nylon nets (Hydrobios, Kiel, Germany) to identify the association of bacterial organisms with zooplanktons and phytoplanktons. Thereafter, 500 mL of filtrate from the 20 μm net, 50 mL from the 55 μm net and 20 mL filtrate from 200 μm net were collected to measure the phytoplankton and zooplankton attached bacteria respectively. Aliquots of the concentrated size fractions, e.g. <20 μm, >20 μm to <55 μm, >55 μm to <200 μm and >200 μm, water samples and plankton samples were transported in an insulated box with ice packs to the central laboratory of National Institute of Cholera and Enteric Disease (NICED) and processed within 24 h of collection.

2.3. Physico-chemical and hydrological parameters

Immediately after sample collection, temperature, pH, conductivity and salinity were measured with a Multi-meter (pH/Cond 340i WTW, Weilheim, Germany). Turbidity was recorded by a portable turbidimeter TD-100 (Eurotech, Singapore) and expressed as Nephelometric Turbidity Units (NTU). Salinity is expressed as Practical Salinity Units (PSU), which for practical purposes is almost identical to g/L or parts per thousand.

2.4. Bacteriological analysis

The samples were analysed for total bacterial count (TBC), total faecal coliform count (TFCC) and cultivable *Vibrio* count (CVC) following standard procedures (Batabyal et al., 2013, 2014b). Briefly, 0.1 mL of each sample (after serial dilution of the raw sample) were spread plated on Nutrient Agar (Becton, Dickinson, USA) for determining TBC; and simultaneously 0.1 mL of each raw sample were spread plated on MFC agar (Hi-Media, Mumbai, India) for TFCC, and thiosulphate citrate bile salt sucrose agar (TCBS; Becton, Dickinson, USA) for CVC determination respectively. In addition to this, for CVC and TFCC determination 10 mL and 100 mL of the raw samples were filtered through sterile 0.22 μm filter paper (Milipore) and the filter papers were put on the TCBS and MFC agar plates respectively. This was followed by an incubation of 18 h at 37 °C for all bacterial determinations, and for 18 h at 44 °C for faecal coliform count. All microbiological enumerations were done in triplicate (Batabyal et al., 2014b).