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Ecological interaction of toxigenic *Vibrio cholerae* in aquatic environment

HEMANT KUMAR KHUNTIA, SURYA KANTA SAMAL, ASHOK KUMAR SARANGI, SUDEEP RANJAN NAYAK, SANTANU KUMAR KAR and BIBHUTI BHUSAN PAL*

Regional Medical Research Centre (ICMR), C. S. Pur, Bhubaneswar - 751 023 (India)

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ABSTRACT

Toxigenic *Vibrio cholerae* is the etiological agent of cholera, an acute dehydrating diarrhoea that occurs in epidemic form in many developing countries. Aquatic ecosystem is the major habitat of toxigenic *V. cholerae*, where it interacts with various abiotic and biotic factors to survive. To acknowledge the seasonal appearance, endemic nature and genetic deviation of *V. cholerae* it is essential to understand the interaction of *V. cholerae* with biotic and abiotic factors in aquatic ecosystem. This article summarizes the ecological interaction of toxigenic *V. cholerae* with different abiotic and biotic factors in aquatic ecosystem during the interepidemic period and their effect in clonal shift due to change of population structure. Suitable salinity, pH, nutrient content, temperature and the presence of aquatic macrophytes, phytoplanktons, zooplanktons, fish, mollusks and crustaceans present in the aquatic ecosystem favors the survival of toxigenic *V. cholerae*. Diversity, relatedness and ecological interaction of toxigenic vibrio population in different habitats can bring changes in genetic make up due to natural stresses. In aquatic ecosystem *V. cholerae* interact with ecofactors for its survival and fluctuation in population structure may cause the dynamics of the diseases.

Key words: Cholera, clone, aquatic, environment, toxigenic, *Vibrio cholerae*.

INTRODUCTION

Cholera has historically been occurred in periodic epidemic form limited to few developing and under developing countries, namely Bangladesh, India, countries in Africa and South America. *V.cholerae* is known to be an autochthonous inhabitant of brackish water and estuarine system. Among more than 200 serogroups of *V.cholerae* so far identified[1], only O1 and O139 have caused epidemic cholera[2-5]. The other serogroups of *V.cholerae* collectively referred to as non-O1 and non-O139 serogroup have not been associated with epidemic but can cause sporadic diarrhoea[6] and ubiquitously distributed in aquatic environment[7]. More than 95% of the strains belonging to serogroups of O1 and O139 produce cholera toxin (CT) which is central to the disease process are designated as toxigenic *V.cholerae*.

It was believed that *V. cholerae* O1 and O139 are able to survive only a few hours in aquatic environment[8]. But later this idea was changed with the observation that the presence of the microorganisms in aquatic environment does not solely dependent on the extent of fecal contamination, as there is no correlation between the presence of fecal coliform bacteria and toxigenic and non toxigenic strains of *V.cholerae* O1 and O139 in aquatic environment[9]. Subsequent hypothesis reported toxigenic *V.cholerae* is an autochthonous member of the microbial flora found in brackish waters typical of estuaries and coastal swamps and can be detected for extended periods in fresh waters, where there is no human fecal contamination[10].

In response to environmental stress in aquatic environments such as low concentrations

of nutrients and low temperatures, *V.cholerae* O1, O139 and Non-O1 and Non-O139 adopt a viable state that enables them to carry out metabolic functions and form colonies but can not be expressed *in vitro* culture[11]. If favorable environmental condition prevails *V.cholerae* can become culturable again. *V.cholerae* O1 & O139 in a viable but non-culturable state has produced clinical symptoms of cholera in volunteers which confirms that it maintains its pathogenicity in aquatic environment despite the inability of the cells to be cultured[11]. Endemic and seasonal nature of cholera depends on the presence of the pathogenic agents in a viable but non-culturable state in an aquatic ecological niche that serves as reservoirs for the agent between the epidemic periods[11]. The objective of this article is to focus the ecological interaction of toxigenic *V.cholerae* associated with biotic and abiotic factors, and its effect on survival and genome of *V. cholerae* in aquatic environment.

It is well understood that to control cholera it is necessary to prevent humans from coming into contact with the natural reservoirs of toxigenic *V.cholerae*. This implies the ability to identify aquatic environmental abiotic and biotic ecological conditions that enable the microorganisms to survive between epidemics. During this period, *V.cholerae* may adopt different conditions to acclimatize the adverse situation which may lead to changes in genetic make up. To determine which aquatic ecosystem can harbor the microorganisms where many phenotypic and genotypic character changes occur it is essential to understand the ecology of *V.cholerae* which will contribute to understand the endemic nature of this disease.

Ecology of toxigenic *vibrio cholerae*

Water

V.cholerae including its toxigenic strains[12] has often been isolated from aquatic environments such as bays, rivers, canals, ponds, ditches and ground water. Cholera transmission takes place primarily through ingestion of water contamination with the feces or vomitus of patients or less frequently with the feces of asymptomatic carriers. Both the toxigenic *V.cholerae* O1 and O139 have been isolated from aquatic environments and are believed to spread primarily by water[13-14].

Nutrients

V.cholerae is facultative anaerobes that can grow in media containing carbohydrates, nitrogen, sulfur, phosphorous and sodium; to obtain such minerals it adheres to sediments. Toxigenic *V.cholerae* O1 and O139 need Na^+ to survive in absence of nutrients. In presence of Na^+, addition of alkaline earth metals Ca^+ and Mg^+, can prolong the survival period of *V. cholerae*[15]. Iron plays a significant role in life process of *V.cholerae* which is absorbed after being chilated by siderspore, vibriobactin.

Salinity

In absence of nutrients, the ideal salinity for growth of toxigenic *V.cholerae* is 25 parts per 1000 *V.cholerae* can grow in aquatic environment of high salinity (45 parts per 1000) if it receives 500µg or more tryptone as a substrate. However *V.cholerae* is able to survive for extended periods and can multiply in fresh water environments in the presence of adequate concentration of nutrients which can meet the minimum need of salinity[16].

Temperature

The ideal growth temperature for toxigenic *V.cholerae* varies between 30^0 and 37^0 C. Toxigenic *V.cholerae* can survive for prolonged period in summer than in the winter[10].

Acidity

Toxigenic *V.cholerae* O1 and O139 can tolerate alkaline environments and is very sensitive to acidity[15]. The optimal pH for survival in 25^0C water is between 7 and 8.5 when salinity is moderate and between 7.5 and 9 when salinity is low.

Biotic factors

Aquatic macrophytes

Toxigenic *V.cholerae* in fresh water aquatic environment adheres to the roots of macrophytes like *Eichhornia crassipes* (water hyacinths) which favors its survival. The pathogenic agent secretes the enzyme mucinase, which is considered to be one of the factors responsible for the virulence of the *V.cholerae* and which degrades the cellular mucilage of plants[17]. Aquatic plants could be environmental reservoirs of the microbes either through a non-specific association or a commensal relationship[17].

Phytoplankton

V.cholerae serogroups target species of sea and fresh water phytoplankton and zooplankton to which it adheres. *V.cholerae* primarily colonizes the oral regions and egg sacks of the planktonic copepods. Reproduction of *V.cholerae* takes place in egg sacks, the digestive system and the chitinous exoskeleton of copepods[18]. The pathogenic agents secrete chitinase, an enzyme that enables it to digest chitin and use it as source of nutrients. Oviposition and expulsion of fecal material by planktonic copepods can expedite dissemination and reproduction of the pathogenic microorganisms in aquatic environments[18]. Toxigenic *V.cholerae* O1 strain can attach the species of green algae and blue green algae that can survive longer due to its ability to derive nutrients from the extra cellular products released by these species[19].

Viable but non-culturable Vibrio mostly found in aquatic environment during the inter-epidemic period due to nutritional defficiency. Culturable Vibrio in aquatic environment adheres to the plankton which withstands the seasonal changes in temperature, salinity, pH and nutrient concentration and that they enter a non-culturable state for a given period as a way to adapt to any adverse situation. Once favorable growth conditions return, *V.cholerae* again adapt its culturable state and pose threat of an epidemic if certain plankton blooms contribute to its reproduction.

Fish, mollusks and crustaceans

In some geographical region, *V.cholerae* has been isolated from shrimps and crabs as well as from Oysters and the intestines of fish. The chitinous surface of the crustaceans provides a suitable substrate for reproduction of the pathogenicity microbes. There has been evidence of existence of an association between the incidence of cholera and the consumption of fish and other raw or undercooked seafood[20].

Aquatic birds

V.cholerae colonizes in certain great blue herons which were detected in its feces but not from water samples collected from bird's habitat. Such aquatic birds can be the carriers of the pathogenic agent and contribute to its overall dissemination[21].

Seasonality

During inter-epidemic period *V.cholerae* passes its lifecycle in aquatic ecosystem closely associated with the micro-niches. The density of *V.cholerae* varies seasonally depending on the significant influence of temperature in aquatic environment[22]. In aquatic ecosystem *V.cholerae* take advantage of micro-niches for the perpetuation of its lifecycle. Population densities of *Vibrio cholerae* are ultimately determined by the adequate presence of niches which in turn is closely triggered by the temperature. In winter season the count of *V.cholerae* remain below detectable levels in plankton and in water sample. Water sediment harbors highest densities of *V.cholerae* immediately after the months when peak appearance is observed in plankton. This suggests the maintenance of lifecycle of *V.cholerae* between the niches with respect to the season[23].

The clinical and environmental divide

After shedding from the intestinal environment of human host, if *V.cholerae* reaches aquatic environment passes its life cycle at different state depending on the types of niches in water under various conditions of temperature, salinity, pH, nutrients and environmental stress. Due to the long term starvation, the loss of toxin producing ability of toxigenic *V.cholerae* was observed in aquatic ecosystem[24] which may be due to natural stress. Although *V .cholerae* O1 has isolated frequently from aquatic environs most of the O1 strains do not produce cholera toxins. However some recent studies have reported the presence of of *ctx*AB gene, encoding cholera toxin in environmental *V. cholereae* O1 and O139 strains[25,14]. During the prolong starvation, *V.cholerae* losses its external morphology of curved rod shaped bacilli and coverts to cocci shape besides the change of physiology of metabolic process. However upon exposure to the favorable condition like intestinal milieu, *V.cholerae* restores the capacity to express the phenotyping and genotyping character.

Genomic profiles and environment interaction

Several examples of microevolution are known to exist in the context of *V.cholerae*. Horizontal gene transfer of O antigen genes has been shown to occur in the generation of O139 and

O37 serogroups and variability in the *tcpA* gene is believed to be caused by homologous recombination[26]. Rapid microevolution occurs among *V.cholerae* strains and that gene flow is not restricted or bottlenecked between environmental and clinical habitats[27]. Environment *V.cholerae* population demonstrates significant geographical isolation but barriers between the clinical habitats and aquatic environment are not significant. In addition to spatial variance, temporal variance is a significant factor explaining total genomic variances among toxigenic *V.cholerae* population[27]. Aquatic environment being the reservoir of toxigenic *V.cholerae*, dynamics of its population contribute significantly to variation in cholera epidemics. Any change in the composition *V.cholerae* population in the aquatic environment that may be driven by seasonal fluctuation in the environment or by introduction of new strains through microevolution or being imported from other system can cause coupled changes in composition and behavior of the clinical population leading to a shift in the dynamics of disease expression in cholera.

Clonal shift

Clonal diversity and continual emergence of new epidemic clone among toxigenic *V.cholerae* has been evidenced by molecular epidemiological studies. Cholera toxin which is responsible for profuse watery diarrhoea is encoded by a lysogenic bacteriophage designated as CTXphi. CTXphi plays an important role for the emergence of new toxigenic clone of *V.cholerae.* The ecosystem comprising of *V.cholerae*, CTXphi, the aquatic environment, and mammalian host offers an understanding of the complex relationship between pathogenesis and the natural selection of a pathogen. Changes in survival capacity of *V.cholerae* combating intestine immunity or stresses in environmental habitats is another potential factor for the institution of clonal shift as has been witnessed the wide spread occurrences of El Tor vibrio in environment of south Africa and Latin America[28].

In general, it is impossible to separate environment factors from biological factors, as can be seen from interrelationships in nature that plays a significant role in the emergence of infectious diseases. In conclusion, *V. cholerae* an environmental inhabitant of brackish, estuarine and marine ecosystem represents an agent of disease that can be dramatically influenced by environmental changes including global environmental changes.

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