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Daily variations in pathogenic bacterial populations in a monsoon influenced tropical environment

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ABSTRACT

Changing climatic conditions have influenced the monsoon pattern in recent years. Variations in bacterial population in one such tropical environment were observed everyday over two years and point out intra and inter annual changes driven by the intensity of rainfall. *Vibrio* spp. were abundant during the monsoon and so were faecal coliforms. *Vibrio alginolyticus* were negatively influenced by nitrate, whereas, silicate and rainfall positively influenced *Vibrio parahaemolyticus* numbers. It is also known that pathogenic bacteria are associated with the plankton. Changes in the abundance of plankton, which are governed mainly by environmental changes, could be responsible for variation in pathogenic bacterial abundance during monsoon, other than the land runoff due to precipitation and influx of fresh water.

1. Introduction

Estuaries are anthropogenic hotspots and are also exposed to natural environmental perturbations. Estuaries influenced by seasonal variations in fresh water flux provide a window for change in the functioning of these ecosystems. The west coast of India experiences the south west monsoon. During the monsoon season estuaries receive increased fresh water discharge and the characteristics of the water body change. Bacterial populations, an important component of the microbial loop, influence food web dynamics and ecosystem functioning. The response of the bacterial population to the changing environment is rapid and an assessment of the health of such an ecosystem benefits from high resolution observations. Virulent pathogenic Vibrio species are expected more frequently in tropical marine environments, since the virulence gene expression seems to increase at elevated environmental temperatures (Mahony et al., 2010). Hence tropical coastal waters with year round temperatures near 30 °C would ensure most favourable growth conditions for these pathogens (Reichardt et al., 2013). Coliform pathogens from faecal contamination have received public and scientific attentions for more than a century and have been regarded as one of the most important indicators for monitoring pathogenic bacteria (Li et al., 2014).

In this study we measured variations in the abundance of Vibrio species (*Vibrio cholerae*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*), faecal pathogens (*Enterococcus faecalis*, *Escherichia coli* O157:H7) and Total coliforms at Dona Paula bay, a semi enclosed bay, located at the mouth of the Zuari estuary (15°27′N, 73°48′E) along the central west coast of India. Zuari estuary receives an average rainfall of ~2500 mm of which 80% occurs during June–August. The study area experiences three seasons (1) South west monsoon (June–September) when the area is inundated with riverine and land runoff due to heavy precipitation and experiences stratification due to high influx of fresh water. (2) A post monsoon season (October–January) which is a recovery period from fresh water domination with an increase in salinity but little change in surface water temperature and (3) a pre monsoon season (February–May) when the water becomes typically marine (Manoj and Unnikrishnan, 2009). A drop in the surface salinity is observed from 35.1 psu in premonsoon to 12 psu during southwest monsoon (Unpublished data).

2. Materials and method

2.1. Sampling

Water samples were collected everyday (between 10:00 and 11:00 h) from July 2009 to August 2011 from Dona Paula bay which is located at the mouth of the Zuari estuary situated on the central west coast of India (Fig. 1). Subsequently, the samples

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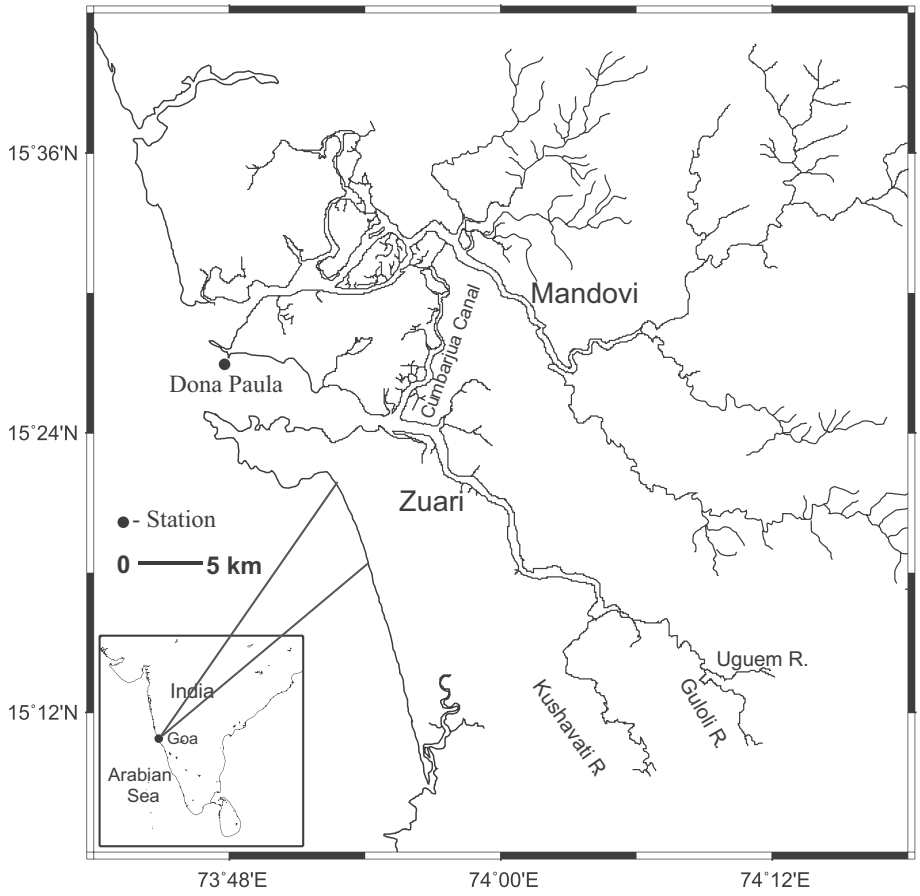

Fig. 1. Map showing Dona Paula bay located at the mouth of the Zuari estuary situated on the central west coast of India.

were diluted as required, spread plated (0.1 ml) on Zobell Marine Agar 2216 (Hi-media) and incubated at (30 ± 2 °C), which was the water temperature of this estuary, for enumerating culturable bacteria. Zobell Marine Agar 2216 allows the growth of culturable, halotolerant and halophilic bacteria.

2.2. Quantification of bacteria

The pathogenic bacteria were quantified using specific media (Hi-media) following the manufacturer's instructions (Hi-media). The culture media were prepared using aged seawater from the study area. The diluted samples were spread plated on Thiosulphate–Citrate–Bile Salts (TCBS) for Vibrio spp. (sucrose fermenting, yellow, entire, raised <2 mm dia colonies were counted as *V. cholerae*, yellow coloured colonies, >2 mm were counted as *V. alginolyticus* and green colour colonies as *V. parahaemolyticus*). Mac Conkey Agar was used for Total Coliforms (Included *E. coli*, *Salmonella* and *Shigella*). Gram negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose fermenting *E. coli* grows as red or pink and may be surrounded by a zone of acid precipitated bile. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless and transparent. Enterococcus Confirmatory Agar was used for *E. faecalis* which grows as blue colour colonies, *Pseudomonas Aeromonas* selective agar for *Aeromonas*/*Pseudomonas* and HiCrome EC0157:H7 selective Agar for *E. coli* O157:H7 (ECO157:H7) which grows as purple colour colonies.

All the plates of specific media were incubated at 37 °C for 24 h and colonies were counted. In order to reduce the uncertainties associated with counting of the pathogenic bacteria, at least 150 pure isolates of each of the pathogenic bacteria were randomly picked from the selective agar and were confirmed using a series of appropriate biochemical tests.

The bacterial species were also confirmed using MALDI-TOF MS Biotyping (MTB), which can be used alone or in combination with other proteomic tools, and has recently become established as a common technique for the detection, identification, and characterisation of micro-organisms (Emami et al., 2012). This method can be complementary to the acquisition of data obtained from 16S rRNA gene sequencing with the added benefit of generating unique biochemical fingerprints for the sub-typing of species.

The culturable bacterial abundance (Total Viable Count, TVC) is expressed as CFU ml^{-1}. The samples to be analysed for total bacterial count (TBC) (including culturable and non-culturable bacteria) were fixed with formaldehyde (final concentration 1–2%; v/v). The quantification of bacteria was performed using acridine orange and epifluorescence microscopy (Daley and Hobbie, 1975) and the values are expressed as cells ml^{-1}.

2.3. Nutrient analysis

Identifying the role of nutrients in the bacterial distribution, water samples were also collected for the nutrient analysis from 1st June 2010 to 31st August 2011. The concentration of different dissolved inorganic nutrients such as nitrate (NO_3–N), nitrite (NO_2–N), phosphate (PO_4–P) and silicate (SiO_4–Si) were determined using an autoanalyser (SKALAR ANALYTICAL SAN PLUS 8505 INTERFACE v 3.31). The rainfall (RF, mm) data were obtained from the Indian Meteorological Department, Panaji, Goa.

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