Spatio-temporal variations in pathogenic bacteria in the surface sediments of the Zuari estuary, Goa, India

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Estuaries are hotspots of anthropogenic activities. The deposition of pathogenic bacteria in the sediment and their re-suspension into the water column are influenced by riverine discharge and tides. The abundance of *Escherichia coli* O157:H7, *Shigella* spp., *Salmonella* spp., total coliforms (TC) and *Vibrio* spp. (*Vibrio cholerae* (VC), *Vibrio parahaemolyticus* (VP), *Vibrio alginolyticus* (VA)) was assessed along with the total bacterial count (TBC) and total viable count (TVC) in surface sediments along the banks of the Zuari estuary, Goa, India. The study was carried out fortnightly for a period of 17 months covering three seasons, i.e. pre-monsoon (PreM), monsoon (MON) and post-monsoon (POM). The spatial and temporal changes in the quality of organic matter were also assessed. The organic matter content was high and rich in carbohydrates and proteins towards upstream sites. The quality of organic matter was influenced by the seasons. *E. coli* O157:H7 was detected only during MON towards the upstream stations. A negative correlation between TC and TBC with salinity was evident indicating the influence of land run-off. The *Shigella* spp. and VA were high towards the mouth of the estuary during PreM. However, during POM, the TVC, TC and VP were abundant towards the upstream and VC were abundant at the mouth of the estuary. Among the *Vibrios*, VP and VA were the most frequently occurring bacteria whereas TC and *Shigella* spp. were dominant among allochthonous pathogens in the sediments irrespective of space and time. In addition to influence of seasons, the sampling time influenced by tidal condition also played an important role in the population dynamics of pathogenic bacteria in the sediments. Future studies should address the interaction of pathogenic bacteria with suspended particles, their transport and survival in the sediments.

Keywords: Coliforms, sediment, estuaries, pathogenic bacteria, *Vibrio* spp.

ESTUARIES and coastal regions are continuously subjected to natural as well as various forms of anthropogenic activities which can change the quality of coastal water rendering it unsafe for recreation or human use[1,2]. The sources of water pollution include point (sewage discharge, domestic wastes and industrial effluents) and non-point sources (agricultural and urban runoff) which bring pathogenic bacteria into the coastal and estuarine waters[3]. The presence of various pathogens (e.g. *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Pseudomonas* spp., *Enterococcus faecalis*, total coliforms, etc.) has been reported in the estuarine and coastal waters of India[4–10]. Most of these pathogens are responsible for waterborne diseases (gastroenteritis, diarrhea, dysentery, typhoid, cholera, food poisoning and wound infection) in humans[3], either due to consumption of contaminated sea food or contact with water[7]. The attachment of allochthonous pathogenic bacteria to the particulate matter in the water column protects them from other environmental and biotic factors. These bacteria are found in large numbers in the sediments rich in organic matter, silt and clay[11,12]. Sediment acts as a reservoir of pathogenic bacteria in estuarine systems[12,13]. There are reports on the re-suspension of coliforms and faecal indicators from sediment bed due to recreational activities, tidal currents and storms[14,15]. Other than anthropogenic activities, the re-suspension of sediment could be another source in adding pathogenic bacteria to the overlying water column. The transport of re-suspended sediment particles is land-ward during flood tide and bay-ward during ebb tide[16].

V. cholerae, *V. parahaemolyticus* and *V. vulnificus* are generally found in marine and brackish environments and are serious human pathogens[17]. Kanungo *et al.*[18] reported that high numbers of cholera outbreaks in India were found in West Bengal, Orissa, Maharashtra and Kerala from 1997 to 2006. According to World Health Organization (WHO), a total of 589,854 cholera cases were found in 58 countries worldwide during 2011 (ref. 19). As sediment acts as reservoir of pathogenic bacteria, monitoring the bacterial load in the sediment can provide stable indicator of their long-term abundance in the water column[20].

Zuari estuary is located along the central west coast of India (15°27.5′N, 73°48′E) and is influenced by the south west (SW) monsoon[21], hereafter referred to as the monsoon. The seasonality in this estuary can be categorized into three seasons: pre-monsoon (PreM; February–May),

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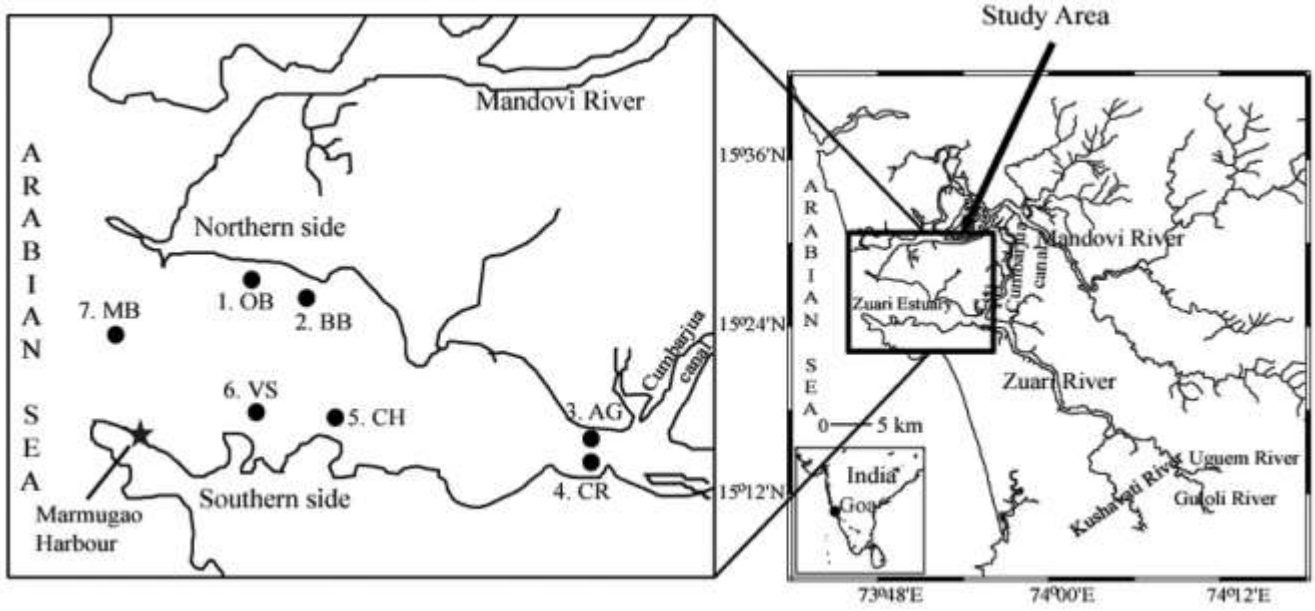

Figure 1. Location of stations in the Zuari estuary, Goa, central west coast of India (station 1, Odxel Beach (OB); station 2, Bambolim Beach (BB); station 3, Agacaim (AG); station 4, Cortalim (CR); station 5, Chicalim (CH); station 6, Vasco (VS); station 7, Marmugao bay (MB)).

Monsoon (MON; June–September) and post-monsoon (POM; October–January)[22]. During monsoon, the run-off is reported to range from 100 to 400 $m^3 s^{-1}$ in the Zuari estuary[23]. The amount of sewage discharged into the Mandovi–Zuari estuaries is estimated to be about 30 million litres per day[24]. Zuari estuary is used for navigation purpose, fishing, shellfish harvesting, transport of ores, discharge of wastes from transport ships, port- and tourist-related activities. Few studies have assessed the abundance of pathogens in the sediments in the midstream of the channel and the bay of the Zuari estuary[6,25–27]. The present study assesses the distribution of pathogenic bacteria in the surface sediments along the estuarine banks which are the active sites of anthropogenic activities. Fortnightly sampling was carried out in the Zuari estuary for 17 months from November 2010 to May 2012, covering all three seasons of the year. The present study also addresses the influence of tidal conditions observed during the sampling period on the distribution of pathogenic bacteria in the sediments.

Materials and methods

Study area

Sampling was carried out in the Zuari estuary located along the central west coast (Goa) of India (Figure 1). Zuari river has a length of 50 km and an average depth of 5 m. The mouth of the estuary is funnel-shaped with a width of ~5 km and narrows down towards the head of the estuary[28]. The estuary remains well mixed during PreM. However, it is stratified during monsoon season with less dense freshwater on the surface and more dense saline water at the bottom. The tides are semi-diurnal, with a height of ~2.3 m during spring tide and ~1.5 m during neap tide[21]. The surface sediment samples were collected from seven selected sites that line the banks of the Zuari estuary and are divided into five categories (Figure 1). Station 1 (Odxel Beach – OB) and station 2 (Bambolim Beach – BB) are categorized as beach influenced stations. Stations 3 (Agacaim – AG) and 4 (Cortalim – CR) are located towards upstream and influenced by the movement of ferry boats and fishing trawlers. The anthropogenic and industrial influence is prominent at station 5 (Chicalim – CH). Station 6 (Vasco – VS) is close to Marmugao port and is situated towards the mouth of the estuary. Tidal influence is high at station 7 (Marmugao Bay – MB) which is located at the mouth of the estuary. The details of the sampling locations are given in Table 1.

Collection of samples

Fortnightly sampling was carried out during post monsoon-I (November–December 2010 and January 2011; POM-I), pre monsoon-I (February–May, 2011; PreM-I), monsoon (June–September 2011; MON), post monsoon-II (December–January 2011/2012; POM-II) and pre monsoon-II (February–May 2012; PreM-II) seasons at the Zuari estuary. Sampling was initiated at station 1 at ~7 : 00 am and concluded at station 7 at ~11 : 00 a.m. The different phases of tides were noticed in the tide table during the sampling period. A slack period was observed before ebb tide during POM-I sampling and ebb tide

Table 1. Details of sampling locations at the Zuari estuary

Station no.	Station name	Station code	Latitude	Longitude
1	Odxel Beach	OB	15°27′03.3″N	73°49′30.1″E
2	Bambolim Beach	BB	15°26′47.4″N	73°50′08.5″E
3	Agacaim	AG	15°24′48.0″N	73°54′17.5″E
4	Cortalim	CR	15°24′33.4″N	73°54′15.3″E
5	Chicalim	CH	15°24′48.2″N	73°50′27.4″E
6	Vasco	VS	15°24′49.5″N	73°49′28.9″E
7	Marmugao bay	MB	15°26′13.6″N	73°47′54.4″E

during POM-II. Flood tide was observed during PreM-I and ebb tide during PreM-II. Monsoon sampling was done during the slack period before flood tide. Thus, except PreM-I season, all other samplings were done either during slack period before ebb or flood tide or during ebb tide. Surface sediment samples were collected by using a van Veen grab and the top ~2 cm of sediment was used to assess the TVC, pathogenic bacteria, total organic matter (TOM), total organic carbon (TOC), total nitrogen (TN), proteins and carbohydrates. Sediment samples to be analysed for total bacterial count (TBC) were fixed with formaldehyde (final concentration 1–2% v/v). The samples were transported to the laboratory in an ice box. The near bottom water samples were also collected using Niskin sampler for analysis of dissolved oxygen (DO), salinity and temperature. The Winkler titrimetric method was used in the estimation of DO[29]. Digital thermometer (EUROLAB) and master refractometer (ATAGO, Japan) were used to measure the temperature and salinity of sea water respectively.

Enumeration of TVC and pathogenic bacteria in the surface sediments: The bacteria in the sediment samples were enumerated using standard spread plate technique. The different selective media were brought from HiMedia (Mumbai) and prepared in distilled water following manufacturer's instructions. One gram of wet sediment was suspended in 10 ml of filtered, autoclaved sea water followed by vigorous shaking to dissociate the bacteria from the sediment. It was then left for 5–10 min for the sediment particles to settle. Subsequently, 1 ml of supernatant was serially diluted in 9 ml of filtered, autoclaved sea water to get 10^1, 10^2, 10^3 dilutions. From each of the dilutions 0.1 ml was plated on Zobell Marine Agar (ZMA) for total viable (culturable) bacteria and incubated at room temperature (28° ± 2°C) for 24 h. Pathogenic bacteria were enumerated by spreading samples on different selective media like MacConkey agar which differentiated *E. coli* (pink/red coloured colonies) from *Shigella*/*Salmonella* species (transparent, colourless colonies). All colonies on MacConkey agar were reported as total coliforms (TC = *E. coli* and *Shigella*/*Salmonella* spp.). Thiosulphate-citrate-bile-salts sucrose (TCBS) agar was used to differentiate *Vibrio* spp. depending on size and colour. *V. cholerae* grow as raised, yellow coloured colonies having diameter <2 mm; *V. alginolyticus* are bigger (>2 mm diameter) in size and produce yellow coloured colonies. However, *V. parahaemolyticus* develop as green coloured colonies on TCBS agar. Xylose-Lysine Deoxycholate (XLD) agar differentiates *Salmonella* (pink coloured colonies with black centre) from *Shigella* (pink colonies) species. Enterococcus confirmatory agar was used for *Enterococcus faecalis* (blue coloured colonies) and general *Streptococci* species (white colonies). Hichrome EC O157 : H7 selective agar was used for *E. coli* O157 : H7 (purple coloured colonies) in which Hichrome EC O157 : H7 selective supplement was added aseptically. All selective media for specific pathogens were incubated at 37°C for 24 h and the counts were expressed as colony forming unit (CFU) g^{-1}. The abundance of *Shigella* and *Salmonella* spp. was determined later to POM-I season (November–December 2010).

Enumeration of TBC in the surface sediments by using flow cytometry: Flow cytometry (BD-Biosciences, USA) was used to analyse formaldehyde fixed sediment samples for TBC. Sediment samples (1 g) suspended in 10 ml of autoclaved sea water were sonicated at 50% power in the water bath sonicator (ultrasonic cleaner, Equitron) for 1 min. This was done thrice to separate cells from sediment particles[30] and was further centrifuged at 3000 rpm for 1 min and the supernatants recovered. One ml of supernatant was passed through BD cell strainer cap to remove larger particles and was subsequently stained with SYBR Green I (Molecular Probes, USA) at 1 : 10,000 final concentration and incubated for 15 min at room temperature in the dark[31]. After incubation, the samples were analysed using a BD FACSAria™ II (BD-Biosciences, USA) flow cytometer equipped with a nuclear blue laser of 488 nm to differentiate green fluorescence excited by blue laser. Emitted light was collected through filter sets of 488/10 band pass (BP) for right angle light scatter (SSC) and 530/30 BP for green fluorescence. Fluorescent beads (1 μm, polysciences) were used as internal standards for calibration. Gating was done against SSC versus green fluorescence (FITC) using BD FACS Diva software and the results are expressed as cells g^{-1} of sediment.

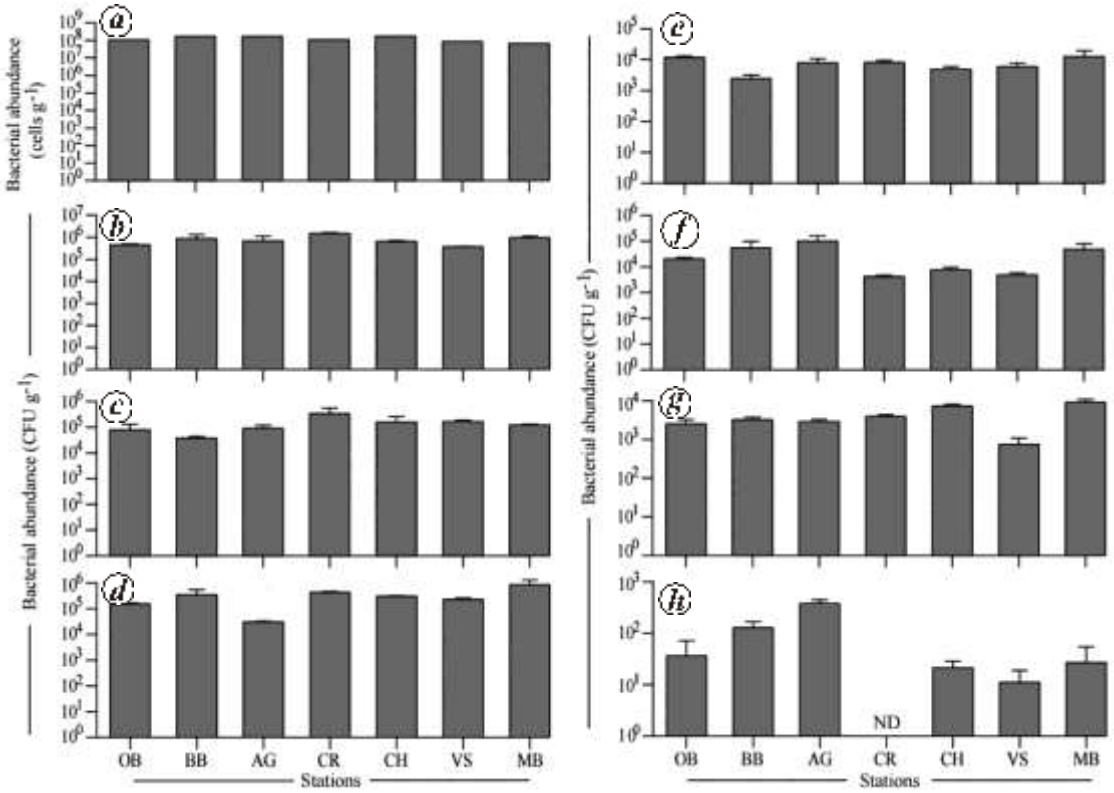

Figure 2. Spatial variations in the (***a***) total bacterial count, (***b***) total viable count, (***c***) total coliforms, (***d***) *V. cholerae*, (***e***) *V. alginolyticus*, (***f***) *V. parahaemolyticus*, (***g***) *Shigella* spp. and (***h***) *Salmonella* spp. in the surface sediments of the Zuari estuary. (These are mean values of bacterial abundance of all 28 fortnightly sampling events). OB, Odxel Beach; BB, Bambolim Beach; AG, Agacaim; CR, Cortalim; CH, Chicalim; VS-Vasco; MB, Marmugao bay; ND, none detected.

Analysis of biogeochemical parameters of surface sediments

Before analysis, the sediment samples stored at –20°C were thawed, dried at 50ºC and ground to fine powder using mortar and pestle. The sedimentary TOC content was determined by wet oxidation with chromic acid as described by El Wakeel and Riley[32]. It was then converted into TOM content by using a factor of 1.724 as described by Bhosle and Dhople[33] and expressed as dry weight percentage (%). Total nitrogen (TN) was determined using CHNS analyser (Vario MICRO Select Elementar) using sulphanilamide as standard and expressed as dry weight percentage (%). The carbohydrate (CHO) content was estimated according to Dubois *et al.*[34] method using spectrophotometer (UV-1800 spectrophotometer, Shimadzu) and glucose as standard. It was expressed as mg g^{-1} of sediment dry weight. The protein (PRT) content was estimated according to Hartree[35] method using bovine serum albumin as calibration standard and the results were reported as mg g^{-1} of sediment dry weight. Sediment samples previously combusted in a muffle furnace at 450°C for 4 h were used as the blanks for biochemical analysis.

Data analysis

A correlation analysis was performed to understand the relationship between different bacterial species (log transformed) and near bottom water salinity, dissolved oxygen and tide. This analysis was done using Statistica 8.0 statistical package.

Results

Physico-chemical parameters of the near bottom water

The near bottom water temperature during the study period varied from 25.7°C to 31.1°C, with maximum temperature during PreM-I. The average salinity fluctuated between 22 and 34. Salinity was low (4) towards the upstream at CR during MON and was high (37) at the mouth (MB) during the PreM-I season. DO in the near bottom water was high during PreM-I and low during POM-II and ranged from 1.55 to 7.83 mg l^{-1}. A strong correlation between DO and tide ($r = 0.67$; $P \leq 0.001$) indicates the influence of tide on DO in the near bottom water.

Table 2. Correlation matrix of environmental parameters with bacterial population in the surface sediments of the Zuari estuary

	TBC	TVC	TC	VC	VA	VP	GS	SH	SL	DO	Salinity	Tide
TBC	1.00											
TVC	**–0.26***	1.00										
TC	0.03	**0.41****	1.00									
VC	–0.08	**0.21***	0.07	1.00								
VA	0.02	**0.35****	**0.30****	0.16	1.00							
VP	–0.04	**0.24***	0.17	**0.36****	**0.51****	1.00						
GS	–0.11	**0.25***	0.05	0.09	**–0.19***	–0.05	1.00					
SH	–0.12	0.11	**0.19***	–0.10	–0.11	0.10	0.13	1.00				
SL	0.11	0.01	0.12	0.06	0.84	–0.02	0.17	–0.03	1.00			
DO	**0.46****	–0.02	**0.26***	–0.10	–0.01	0.16	0.00	0.12	**0.22***	1.00		
Salinity	**–0.26***	0.01	**–0.21***	**0.23***	–0.01	–0.09	0.10	–0.11	–0.12	**–0.44****	1.00	
Tide	**0.38****	–0.03	**0.25***	–0.03	–0.05	**0.25***	–0.12	0.15	–0.15	**0.67****	**–0.34****	1.00

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level. Significant correlations are highlighted in bold.
TBC, total bacterial count; TVC, total viable count; TC, total coliforms; VC, *V. cholerae*; VA, *V. alginolyticus*; VP, *V. parahaemolyticus*; GS, general *Streptococci* spp.; SH, *Shigella* spp.; SL, *Salmonella* spp.; DO, dissolved oxygen.

Spatio-temporal variations of the bacterial populations (TBC, TVC and pathogenic bacteria) in the surface sediments

The average abundance of TBC in the sediment was high (1.77×10^8 cells g^{-1}) at CH, a lower middle estuarine station and minimum (6.54×10^7 cells g^{-1}) at the mouth of the estuary (MB) (Figure 2 *a*). The abundance of TBC was high during MON, low during POM-I (Figure 3 *a*) and was influenced positively by near bottom water DO ($r = 0.46$; $P \leq 0.001$) and negatively by salinity ($r = –0.26$; $P \leq 0.006$). The tide showed a significant influence ($r = 0.38$; $P \leq 0.001$) on the distribution of TBC (Table 2). The TVC ranged from 3.64×10^5 to 1.48×10^6 CFU g^{-1} with maximum abundance towards the upstream at CR during POM-I season (Figures 2 *b* and 3 *b*).

The average abundance of TC (3.34×10^5 CFU g^{-1}) and general *Streptococci* spp. (GS) (1.22×10^3 CFU g^{-1}) was high during POM-I at CR (Figure 2 *c*). TC were influenced positively by bottom water DO ($r = 0.26$; $P \leq 0.006$), tide ($r = 0.25$; $P \leq 0.009$) and negatively by the salinity ($r = –0.21$; $P \leq 0.030$; Table 2). The average abundance of TC decreased from POM-I to POM-II season (POM-I > PreM-I > MON > POM-II; Figure 3 *c*). *E. coli* O157 : H7 was found towards upstream stations only during MON (Supplementary Table 1) and were high (3.90×10^3 CFU g^{-1}) at CR. The *Shigella* spp. and VA were high towards the mouth of estuary during PreM-II, whereas *Salmonella* spp. were high towards the upstream during PreM-I. The abundance of VC ranged from 3.02×10^4 to 8.48×10^5 CFU g^{-1} and was high at MB, the mouth of the estuary during POM-I (Figure 2 *d*) and influenced positively by near bottom water salinity ($r = 0.23$; $P \leq 0.015$). However, VP were high (9.85×10^4 CFU g^{-1}) towards upstream at AG (Figure 2 *f*) and influenced by tide ($r = 0.25$; $P \leq 0.009$; Table 2). Seasonal variation showed high abundance of VC and VP during POM-I (Supplementary Table 1). The percentage occurrence of pathogenic bacteria in the surface sediments showed dominance of VP (88.8%) and VA (80.9%) among autochthonous *Vibrio* spp. and TC (79.3%) and *Shigella* spp. (69.6%) among allochthonous pathogens (Table 3).

Biogeochemical composition of the surface sediments

The upstream stations (AG and CR) and anthropogenically influenced lower estuarine station (CH) were characterized by relatively high content of TOM, TOC and TN when compared to stations towards the mouth of the estuary (Table 4). TOM, TOC and TN in the surface sediments ranged from 0.08% to 6.98%, 0.05% to 4.05% and 0.00% to 0.35% respectively at the Zuari estuary (Table 5). High quantity of TOM and TOC was observed during MON. The spatial and seasonal distribution indicated that the concentration of CHO was more than PRT in the sediments. The concentration of PRT and CHO varied from 0.13 to 16.1 mg g^{-1} and 0.45 to 18.2 mg g^{-1} dry weight of sediment respectively (Table 5).

Discussion

Bacteria in the estuarine and marine environment play a significant role in biogeo-chemical processes, nutrient cycling and organic matter degradation[36]. The present study showed high abundance of TBC in sediments of CH, a lower middle estuarine station influenced by anthropogenic activities. This site is actively used for ship building workshops, yards and subjected to various land-based anthropogenic activities[37]. A recent study on microbial community structure in the surface sediment of CH in the Zuari estuary reported dominance of *Gammaproteobacteria* which includes members of potential pathogens such as *Enterobacteriaceae*, *Vibrionaceae* and

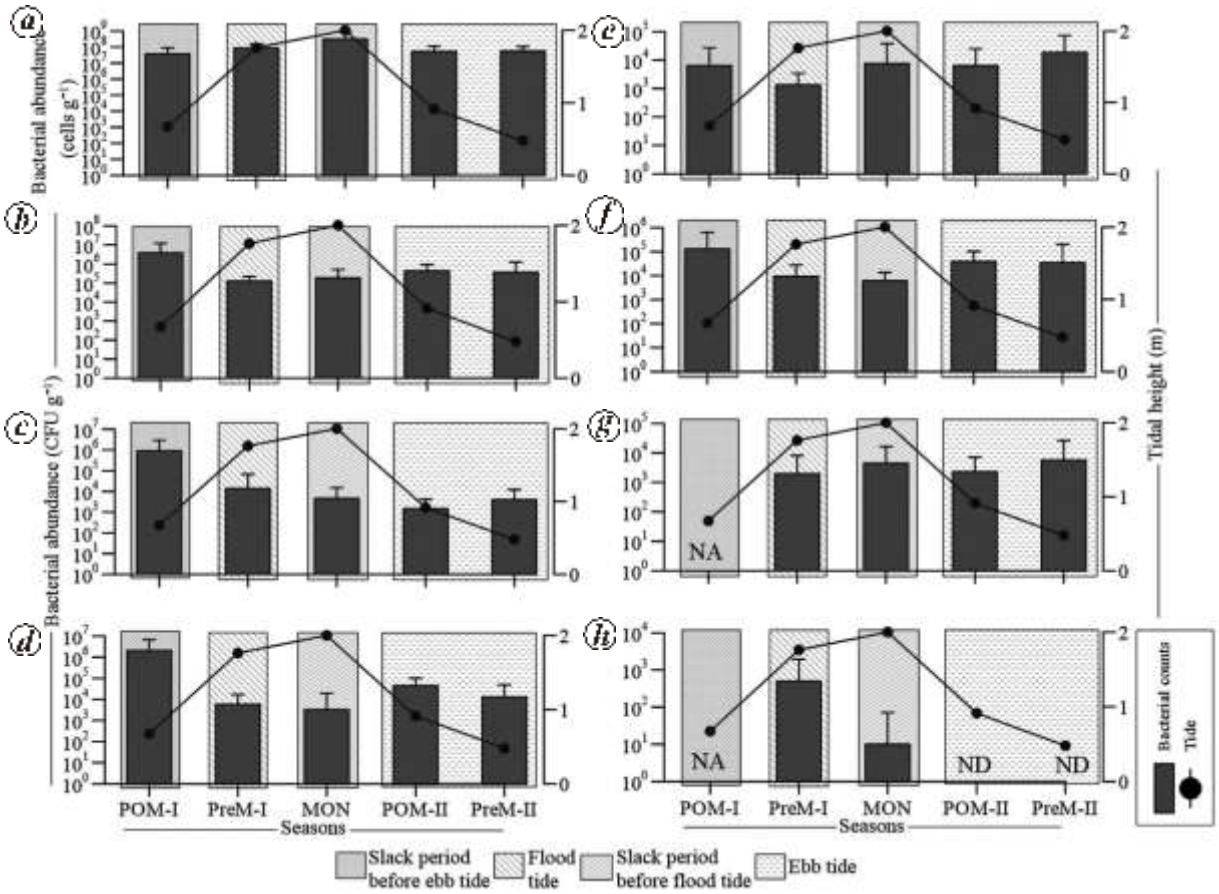

Figure 3. Seasonal variations in the (***a***) total bacterial count (TBC) (***b***) total viable count (TVC), (***c***) total coliforms (TC), (***d***) *V. cholerae*, (***e***) *V. alginolyticus*, (***f***) *V. parahaemolyticus*, (***g***) *Shigella* spp., (***h***) *Salmonella* spp. in the surface sediments of the Zuari estuary during different tidal phases. (NA, not analysed; ND, none detected). Tidal data for study period was obtained from tide table.

Table 3. Percentage (%) occurrence of different pathogens in the surface sediments of the Zuari estuary

Bacteria	Number of samples analysed	Positive occurrence	Overall occurrence (%)
TC	188	149	79.3
VC	188	128	68.1
VA	188	152	80.9
VP	188	167	88.8
EC	188	8	4.3
SH	125	87	69.6
SL	125	10	8.0
GS	188	39	20.7

TC, total coliforms; VC, *Vibrio cholerae*; VA, *V. alginolyticus*; VP, *V. parahaemolyticus*; EC, *E. coli* O157:H7; SH, *Shigella* spp.; SL, *Salmonella* spp. and GS, General *Streptococci* spp.

Pseudomonadaceae[38]. In general, high abundance of TBC was observed during MON. Large amount of fresh water discharge from catchment area during MON could be one of the sources that brings in land run-off rich in bacterial load and anthropogenic input. Zuari estuary receives maximum sediment discharge during peak rainfall[22]. Recently, Shynu *et al.*[39] studied $d^{13}C_{org}$ in the sediment and reported terrestrial organic matter to be the dominant component in the lower region of the Zuari estuary during MON. The ratio of TOC/TN in the sediment has been used to distinguish the autochthonous or marine organic matter from allochthonous or terrestrial input. The protein-rich algae and plankton are characterized by low C/N ratio (4–10) than terrestrial land plants (>20) which are rich in cellulose[40]. TOC/TN ratio in the present study was 15.44 during MON which indicates mixed input of terrestrial and marine derived organic matter in the surface sediments. This could be another possibility for high abundance of TBC. Mahalakshmi *et al.*[41] also observed high density of heterotrophic bacteria in the sediment and water column during the MON at the Uppanar estuary located on the south east coast of India.

The TVC, TC and general *Streptococci* spp. were high at CR which is located at the entrance of the channel near the junction between bay and upstream region of Zuari river, is shallow in depth and is influenced by tidal currents, waves, movement of fishing trawlers and ferry boats. Previous study by Dessai and Nayak[42] reported that sediments in this region are rich in silt and clay content due to deposition of fine particles during low energy

condition and colloidal aggregates during estuarine mixing. The mouth region is rich in sand owing to intense tidal currents which cause the transfer of fine grain particles away from the mouth. This station was also characterized by high TOM, TOC, TN, proteins and carbohydrates which possibly favoured high abundance of TVC, TC and general *Streptococci* spp. A parallel study on the sediment texture reported that the stations towards the upstream are rich in silt and clay (54.6%) content when compared to those located at the mouth (19.3%) of the estuary (Desai and Atchuthan; personal observation). Perkins *et al.*[11] also reported high abundance of pathogenic bacteria in organic matter, silt and clay rich sediments at the Conwy estuary, UK. The attachment of faecal coliforms to the fine particles increases their viability and transport to the sediment bed[43]. The fine grained sediments with large surface area have more adsorptive capacity for organic matter[44] and influence the accumulation of organic matter and bacteria. TOC/TN ratio indicated input of both autochthonous and terrestrial organic matter at CR which supported high abundance of allochthonous coliforms. The dominance of carbohydrates over proteins and low PRT/CHO ratio (<1) indicates the detrital heterotrophic nature of the environment[45]. The abundance of TC in the present study ranged from none detected (ND) to 88.7×10^5 CFU g^{-1} and was almost similar to the previous study (ND – 47.7×10^5 CFU g^{-1})[25]. The abundance of *E. coli* was 2-folds lower (ND – 3.90×10^3 CFU g^{-1}) than the previous study[25] (ND – 6.4×10^5 CFU g^{-1}). However, their study did not include the influence of tides. The flood tide and ebb tide to a certain extent cause decrease in the abundance of pathogenic bacteria from the sediment. A recent study reported that depending on the riverine discharge and tidal amplitude interplay sediment re-suspension, mediated increase in suspended particulate matter (SPM) significantly regulates bacterial population in the Zuari estuary[27]. The re-suspension of sediment affects the quality of overlying water by addition of SPM, organic matter and pathogens which are hazardous to human health. The assessment of pathogenic bacteria in the sediment can improve our understanding of the behaviour, fate and mitigation of potential pathogens in the sediment as well as water body[46].

The abundance of TVC, TC, general *Streptococci* spp. and VP was significantly higher during slack period before ebb tide of POM-I than ebb tide of POM-II. This variation in TVC and pathogenic bacterial abundance is attributed to the nature of sediment as well as tidal conditions observed during POM. It is well known that re-suspended particles settle to the sediment bed during slack period before starting of ebb tide. They get re-suspended from the sediment with ebb currents, remain suspended to a certain degree and then settle to the bottom[47]. The pathogenic bacteria attached to suspended particles increase their downward flux to the bottom sediment[11,48]. This is the possible reason for high abundance of coliforms, VP and general *Streptococci* spp. in the sediments towards the upstream station during POM. The negative influence of near bottom water salinity on the abundance of total coliforms in the sediment indicates their extraneous input into the sediment. The presence of the pathogenic strain, *E. coli* O157 : H7 in the sediment towards the upstream stations only during MON season can be attributed to land run-off and sewage input. Nagvenkar and Ramaiah[26] also reported the influence of land run-off and domestic sewage input on the abundance of fecal coliforms in the Zuari–Mandovi estuaries.

High abundance of *V. cholerae* was observed at the mouth of the estuary during slack period prior to ebb tide of POM. A recent study also reported high numbers of VC in the water column at the mouth of the Zuari estuary[27]. Their abundance was influenced by SPM, nutrients and salinity. The ratio of TOC/TN was low (8.71) at the mouth of the estuary, indicating contribution of phytoplankton which could be a possible reason for high abundance of VC, as *Vibrio* spp. are associated with plankton population[49]. The abundance of *Shigella* spp. and VA was high towards the mouth of the estuary during ebb tide of the PreM-II season indicating their deposition during ebb tide. However, *Salmonella* spp. were high towards the upstream during flood tide of PreM-I. The flood tide currents cause re-suspension of sediment bed and transfer particles towards land. During ebb currents particles move towards the sea and settle to the bottom sediment[47,50]. An earlier study in this estuary had also pointed out that the effluents containing high numbers of pathogenic bacteria accumulate towards downstream during low tide-period[6].

Overall, the present study showed that the percentage (%) occurrence of VP and VA was high in the sediments as they are autochthonous to the estuarine and marine ecosystems[17]. High prevalence of coliforms and *Shigella* spp. among allochthonous pathogens can be attributed to their adaptability and survival in the sediment. Pathogenic bacteria adsorbed to sediment particles protect themselves from UV light, phage attack and protozoan grazing and hence survive for longer time in the sediment[11–13,46]. The fecal indicator bacteria can even proliferate and regrow in organic matter and fine particle-rich sediments[51]. Moreover, VP emerged as a dominant bacterium among all pathogens in the sediment during the study period (Table 3). VP can tolerate wide range of salinities and is able to use large numbers of substrates for growth[52,53]. VP are found to be associated with copepods and play a significant role in nutrient cycling by mineralization of chitinous material with help of chitinase enzymes[17,53]. Also, it has been reported that VP are associated with phytoplankton and their growth is influenced by decaying planktonic cells[54]. Zuari estuary is productive during non-monsoon seasons and subjected to heterotrophy during monsoon due to large input of

Table 4. Spatial variations in organic matter parameters in the surface sediments of the Zuari estuary

Parameters	OB	BB	AG	CR	CH*	VS	MB
TOM (%)	1.03 ± 0.79	1.06 ± 0.76	2.64 ± 1.43	4.25 ± 1.73	2.57 ± 2.33	2.09 ± 0.77	1.05 ± 1.04
TOC (%)	0.60 ± 0.46	0.61 ± 0.44	1.53 ± 0.83	2.46 ± 1.00	1.49 ± 1.35	1.21 ± 0.45	0.61 ± 0.61
TN (%)	0.04 ± 0.04	0.07 ± 0.06	0.15 ± 0.11	0.18 ± 0.11	0.09 ± 0.07	0.07 ± 0.03	0.07 ± 0.09
TOC/TN	15.0	8.71	10.20	13.67	16.56	17.29	8.71
PRT (mg g^{-1})	1.37 ± 1.12	2.25 ± 1.60	5.97 ± 3.46	9.95 ± 3.22	5.32 ± 4.14	4.27 ± 1.36	1.92 ± 3.22
CHO (mg g^{-1})	1.91 ± 1.12	3.33 ± 2.54	8.12 ± 4.83	11.8 ± 4.67	7.06 ± 5.70	4.51 ± 2.17	2.87 ± 4.07
PRT/CHO	0.72	0.68	0.74	0.85	0.75	0.95	0.67

OB, Odxel Beach; BB, Bambolim beach; AG, Agacaim; CR, Cortalim; CH, Chicalim; VS, Vasco; MB, Marmugao bay; *Anthropogenically influenced; TOM: Total Organic Matter; TOC, total organic carbon; TN, total nitrogen; PRT, proteins; CHO, carbohydrates.
These are station wise average (± standard deviation) values of sedimentary parameters including different seasons (PreM, MON and POM).

Table 5. Range and average concentration of organic matter parameters during different seasons in the surface sediments of the Zuari estuary

	POM-I			MON		POM-II		PreM-II	
Parameters	Min–Max	Avg ± SD	PreM-I	Min–Max	Avg ± SD	Min–Max	Avg ± SD	Min–Max	Avg ± SD
TOM (%)	0.40–6.87	2.17 ± 2.36		0.08–6.98	2.39 ± 2.04	0.59–4.06	1.83 ± 1.16	0.18–4.72	1.81 ± 1.46
TOC (%)	0.23–3.99	1.26 ± 1.37		0.05–4.05	1.39 ± 1.18	0.34–2.35	1.12 ± 0.67	0.10–2.74	1.05 ± 0.85
TN (%)	0.00–0.05	0.02 ± 0.02	No samples	0.00–0.20	0.09 ± 0.07	0.03–0.19	0.10 ± 0.08	0.00–0.35	0.10 ± 0.10
PRT (mg g^{-1})	0.42–12.9	4.17 ± 4.84		0.13–16.1	4.41 ± 4.15	1.33–11.4	5.34 ± 3.57	0.28–10.9	3.98 ± 3.54
CHO (mg g^{-1})	0.86–18.6	5.84 ± 6.75		0.45–18.2	5.65 ± 5.21	1.61–14.4	6.49 ± 4.59	0.48–13.6	5.11 ± 4.54

Min, Minimum; Max, Maximum; Avg, average; SD, standard deviation.

terrestrial organic matter and nutrients through land runoff[22,55]. Estuaries play a significant role in trapping and modification of autochthonous and terrestrial organic matter[56]. The present study showed mixed input of marine and terrestrial organic matter in the surface sediments. Low PRT/CHO ratio (<1) indicates the presence of detrital organic matter. High prevalence of VP in the surface sediments suggests their role in the mineralization of detrital organic matter derived from marine and terrestrial inputs.

Conclusions

The present study revealed a significant role of organic matter content, seasons and tide on the distribution of pathogenic bacteria in surface sediments along the banks of the Zuari estuary. The organic matter was of mixed origin (autochthonous and terrestrial) towards upstream and autochthonous at the mouth of estuary and had profound influence on the distribution of pathogenic bacteria in surface sediments. The low (<1) PRT/CHO ratio suggests utilization of proteins and a heterotrophic environment in the sediment. High abundance of pathogenic bacteria in the sediment during slack period before ebb tide indicates their deposition in the sediment. VP and VA were the more frequently occurring bacteria among autochthonous, whereas TC and *Shigella* spp. were dominant among allochthonous pathogens in the sediment. Future studies should address the interaction of pathogenic bacteria with suspended particles, their transport and survival in the sediment which will help in understanding their ecology.

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ACKNOWLEDGEMENTS. We acknowledge the facilities and support provided by the Director, National Institute of Oceanography. We thank Dr Dattesh Desai and other colleagues of Biofouling and Bioinvasion Division (BBD) for their help during sampling. This work was supported by grants under Ballast Water Management Programme, India (BAMPI-India), Ministry of Shipping and DG Shipping, Government of India and CSIR funded Ocean Finder Program. This is a NIO contribution No. 6051.

Received 18 November 2016; revised accepted 9 May 2017

doi: 10.18520/cs/v113/i09/1729-1738