Environmental Drivers of the Seasonal Prevalence of Enteropathogenic Bacteria in the Gulf Ecosystem of Gujarat, India: Unravelling Its Diarrheal Enigma

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Abstract: - Recurrent episodes of water borne diarrheal outbreaks is the pressing public health crisis in the Gulf of Khambat (GoK), Gujarat and its adjoining area. We aimed to identify seasonality of environmental signatures associated with the dynamics of Gulf enteropathogens and related health implications. A yearlong sampling data has been generated from the five sites across the GoK of high anthropogenic burden and contrasting hydrological settings. Hydro-chemical characteristics of water samples and bacteriological indices were analysed on field and laboratory condition respectively and robust statistics was applied to assess their interrelationship and associated health risks. Annual variation of hydro-chemical indices viz. temperature 26.4ºC–36.7ºC, pH 7.51–8.44, salinity 28.3–36.7 mS/cm, turbidity 3.6–995 NTU and Dissolved Oxygen (DO) was recorded in the water samples. Extremely high turbidity (70.5-995) along with high salinity (28.3-34.8) is found to be the unique characteristic feature of Gulf water, which facilitates the abundance of various enteropathogenic bacterial species, specially the clinically important ones like *V. cholerae*, *V. alginolyticus* and *E. coli*. Surprisingly, an explosive annual preponderance of *V. alginolyticus* could be observed throughout all the study sites and existence of *V. cholerae* is restricted only in the high turbid water (>500NTU) of Gulf. Detection of *E. coli* is also a significant report from this high saline habitat, indicating faecal contamination. Seasonality of Vibrios and coliforms are highly influenced by precipitation rate and turbidity of water ($p<0.05$). DO level is found to be another crucial marker which regulates distribution of enteropathogens across the Gulf water as well as indicates the pollution level of Gulf attributed to environmental health risk. This is the first report of a longitudinal study of enteropathogenic load in GoK and its direct relationship with diarrheal incidence profile, which adds valuable knowledge for the formulation of 'bio-environmental tool' for effective water quality monitoring and disease prevention.

Key-Words: - Gulf water, environmental factors, Vibrios, *E. coli*, *V. alginolyticus*, Seasonality, Diarrhoea.

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1 Introduction

Diarrheal diseases is an increasingly pressing public health concern with an estimated death toll of about two million per year and ranking third amongst all the infectious diseases around the world [1,2]. Death is caused by depletion of body fluids resulting in profound dehydration in patients. Immediate rehydration and replenishment of body fluid may be achieved by administration of oral rehydration solution (ORS), which is effective to save lives during epidemics [3]. Transmission primarily or exclusively occurred by the fecal-oral route by means of healthy water scarcity, consumption of contaminated seafood, unhygienic lifestyle, and lack of sanitary practices [4-7]. UNICEF estimated that about 88% of diarrhoea-associated mortality is attributable to poor sanitation and hygiene practices [8]. Therefore, it has the highest impact on underprivileged communities of developing countries like India, where adequate disease surveillance and mitigation management are scarce [9]. Diarrhoea has been a recurring problem in India for over a century now, with sporadic as well as large outbreaks occurring around the year [10, 11]. In India, diarrhoea took more lives than any other water-borne diseases and became endemic in various provinces of different eco-hydrological settings including the deltaic peninsula of the Indo-Gangetic Plain, where the riverine-estuaries have been established as a potential reservoir for effective disease transmission [12,13] and simultaneously at a distance of ~2000Km, the Gulf ecosystem of Gujarat also harbor various enterobacterial strains and presumed to act as another potential reservoir of enteropathogens [14].

1.1 Background to the study

The coastal community of Gujarat, the largest maritime state of India experiences escalating episodes of acute diarrheal outbreaks during the last decade [15]. The state encompasses the longest coastline of ~1600Km and two adjoining Gulfs, situated on the western flank of India, which severely affects its public health status with the high level of saline water ingression into the community water table [16]. Lack of freshwater availability and drinking water scarcity engrossed almost 2/3 part of the state [17]. Consequently, this condition accelerated water-borne infections like diarrhoea across the province. Bhavnagar, being situated on the western bank of the 'Gulf of Khambat (GoK)' and Surat on its eastern bank, have a direct impact of salinity and rocky terrain on its groundwater system, which deficits accessibility of healthy water for its thickly populated community [18]. They are also among the top five districts which experience maximum cases of acute diarrheal diseases (ADD) annually (IDSP) and several sporadic diarrheal outbreaks have been documented from the urban and rural settings located near the Gulf [19,20]. The Gulf water is being regularly used by the nearby inhabitants for bathing, performing sacred rituals and other household chores, which escalates the risk of pathogenic invasion manifold from the Gulf water into the community settings.

Additionally, freshwater scarcity looms large in the Gujarat province due to a lack of adequate freshwater resources. An ambitious project funded by the Gujarat government has been formulated to create a freshwater reservoir by constructing a dam across the Gulf of Khambat, aiming to fulfil the irrigation, domestic, and industrial water requirements of the province. Anticipating the conversion of part of the GoK area into a freshwater lake, it is assumed that there will be a huge impact on the existing microflora and faunal diversity in the gulf [14]. Therefore, estimation of the halotolerant enteropathogenic load of the gulf water became another essential prerequisite for understanding the impact of salinity transition over a period of time on the diarrheal paradigm.

1.2 Problem statement

Considering the catastrophe of recurrent epidemics of diarrhoea and cholera in this province, elucidation of its community transmission mechanism and the role of environmental strains involved in the emergence of pathogenic progenies, persistence and spread of potential epidemics are of paramount importance. Simultaneously a great amount of research efforts is also needed, to comprehend the hydroclimatic factors regulating seasonal dynamics of these virulent enteropathogens in their natural niche for the understanding of its ecology and disease-causing ability.

Earlier studies conducted thus far from a similar ecosystem reported the bacterial dynamics associated with coral reefs across the Gulf of Mannar and Gulf of Kutch [21, 22], without attempting to address or focus on pathogenic bacterial dynamics of gastrointestinal relevance vis-à-vis human health impact. Studies specifying toxigenic Vibrios of the Gulf of Mexico were explored towards understanding the environmental factors linked with Vibrio dynamics [23] but didn't attempt to correlate with public health implications. In another interesting study from 'Sundarban mangrove' (largest mangrove of its kind in the world), altogether different ecological niche, seasonal *V. cholerae* dynamics has been established in environmental settings of high saline mangrove and brackish water flowing to inland low saline condition [24]. However other halophile diarreagenic pathogen were beyond the study purview. With this background the environmental setting of the Indian Gulf is quite different and its impact on the array of potentially pathogenic enterobacterial entities along with associated health risks on the adjacent coastal community has never been explored. Thus, we aimed to investigate the abundance and distribution of diarrheal pathogens across the entire gulf ecosystem as well as its adjoining seawater settings, which distinctly lacks but direly needs a longitudinal comprehensive study for the understanding of its unsolved diarrheal enigma. Simultaneously, we have also conducted a seasonal study to elucidate the role of environmental stimulants on the seasonal dynamicity of diarrheal pathogens. The findings will also help to provide a fundamental knowledge base for developing an effective disease forecasting system to reduce human vulnerability.

2 Material and methods

2.1 Sampling

2.1.1 Study Foci:

The present study was conducted in the Gulf of Khambat (Gok), which is a south to north penetration of the Arabian Sea on the western shelf of India between the Saurashtra peninsula and Bhavnagar district of Gujarat [18]. Five sampling sites have been selected across the Gulf coast with high anthropogenic burden and contrasting hydrological settings, which are separated by ~60Km distance from the adjacent one. Amongst them 'Koliyak' site is situated on the northern bank of GoK with high anthropogenic intervention from the nearby capital city of Bhavnagar. 'Alang' site is located towards the downstream direction from Koliyak and harbor world's largest ship wreckage yard and thus experiences huge oil spillage [25]. 'Mahuva' site is a thickly populous coastal town and placed in the transition zone of the Gulf and seawater, where the Gulf is very wide and deep. Both 'Ghoghola' and 'Veraval' sites are located on the Arabian coast and are inundated with huge crowds all over the year because of nearby tourist destinations (Fig. 1).

2.1.2 Sampling strategies

Marine water samples were collected on monthly mode from the shoreline of the aforementioned five sampling sites using a sterile metallic bucket (5 L) from 1 m. underneath the water surface, pooled and subsequently aliquoted into 500-mL sterile glass bottles and transported to the central laboratory in dark Styrofoam boxes by maintaining ambient temperatures [24, 26]. The collection periods have been settled as winter (December–February), summer (March-May), monsoon (June–September), and post-monsoon (October–November) to embrace the effect of seasonal changes.

2.2 Hydro-chemical study of water samples

Immediately after sampling, water temperature, pH, salinity and Dissolved Oxygen (DO) were measured *in situ* by directly placing Rugged LDO probes into the flowing water body and turbidity was measured with a portable turbidimeter (TD-100, Eurotech, Singapore) inside the laboratory setup.

2.3 Bacteriological analysis

Enumeration of bacteriological load viz. Total Bacterial Count (TBC), Total coliform count (TCC), Total *E. coli* count (TEC) and Cultivable Vibrio Count (CVC) in the water samples were carried out by respective conventional protocol [27, 28]. Briefly, 0.1 mL of each sample (diluted or concentrated) were spread plated on Nutrient Agar (Becton, Dickinson, USA) for TBC; Chromocult Coliform Agar (Merck, Darmstadt, Germany) for TCC and TEC; and Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS; Becton, Dickin- son, USA) for CVC determination, respectively followed by overnight incubation at 37^{o}C. All bacteriological enumerations were done in triplicate. Subsequently, the conventional PCR method was employed to identify suspected Vibrio species viz. *V. cholerae*, *V. parahaemolyticus*, *V. alginolyticus*, *V. mimicus* and *V. vulnificus* [29] and established biochemical screening technique was applied to isolate *E. coli* [30].

2.4. Data processing and statistical validation

Statistical box plots of environmental and bacteriological datasets were constructed using 'Origin software' (V.08; Origin Lab Corporation) representing small squares as mean, horizontal lines as median, vertical bars as standard deviation, and outliers are indicated by highest or lowest points. Spatial distribution of hydro-chemical parameters across the study sites and annual disposition of bacterial load extrapolated with disease burden were ordinated by MS Excel 2013 and Origin Lab V.08. Perason's correlation coefficient at statistical significance level amongst environmental and bacteriological markers was deduced using XLSTAT 2020 statistical packages.

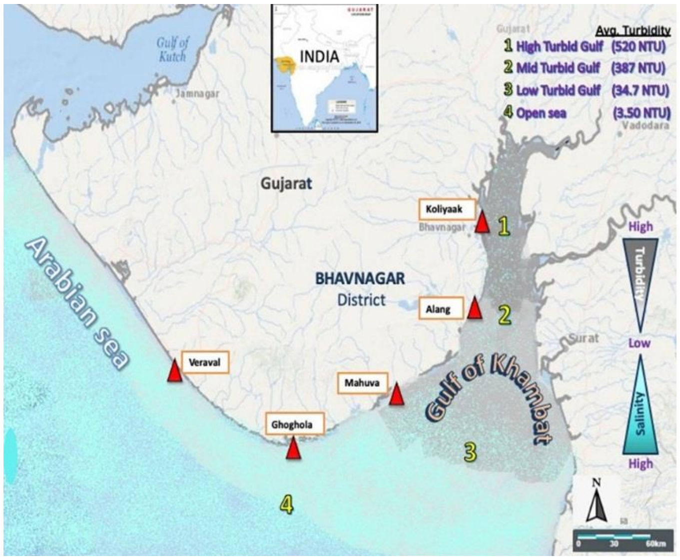


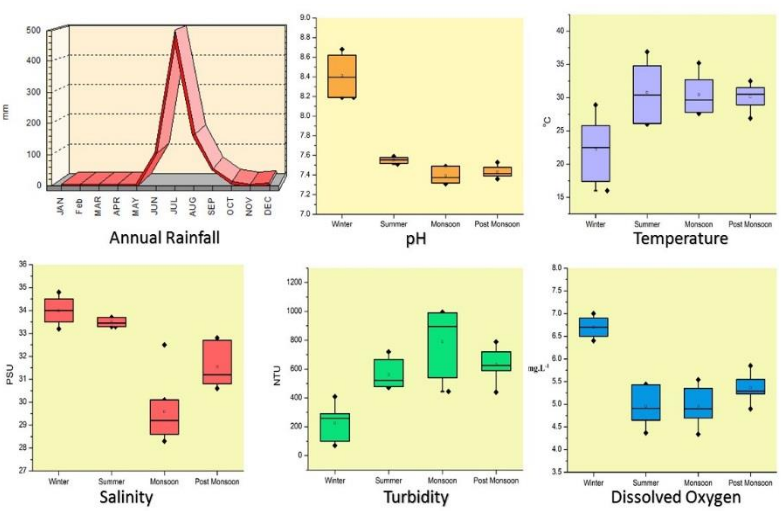
Fig. 1 Map of Study Sites

3 Results and Discussion

3.1 Seasonality of environmental variants

The water temperature of all the investigated samples, collected during the sampling period, varied between 17.5°C to 35.5°C, showing the highest peak in summer and lowest in winter months. However, temperature fluctuations across the study sites are insignificant. Water pH was ranged between 7.31-8.68 with maximum pH obtained in winter and minimum in monsoon period (Fig. 2). The monsoonal drop of pH level is the result of dilution of water body aided by heavy rainfall and subsequent inflow of organic matters from the shores. In the Open sea (Ghoghola and Veraval sites) pH level rises up to 8.44, whereas it remains low (7.56± 0.19) inside the Gulf sites of Koliyak and Alang (Table 1). Discharge of organic debris carried by various riverine estuaries as well as disposal of agricultural and industrial runoffs into the Gulf, possibly influence the reduction of alkalinity inside the Gulf area. The rainfall data was accessed from the archive of the Indian Meteorological Department which depicts that the rate of precipitation remains low throughout the year except for July, only when the Gulf experiences heavy rainfall [31].

Distinct variations of salinity and turbidity level have been detected across the stretch of the Gulf of Khambat. The salinity level remains consistently high (28.3-36.7 PSU) throughout all the seasons. However, its spatial distribution depicts a gradual decrease in average salt density as seawater enters the Gulf. The highest salinity of seawater was recorded at 'Ghoghola' (36.63± 0.04PSU) and 'Veraval' (36.02 ± 0.2 PSU) sites. After that, it successively decreases at the transition zone of 'Mahuva' (34.95 ± 0.05 PSU) and depletes to salinity minima at the deep inside of the Gulf site Koliyak (32.8 ± 1.1 PSU), where freshwater drained by adjacent rivers dilutes the Gulf water (Table 1).Contrastingly, the maximum turbidity was observed at the 'Koliyak' site (520 ± 172 NTU), where a huge amount of mud water is discharged from the adjoining rivers into it (Fig. 3). Thereafter, turbidity level decreases a little at the 'Alang' site (387 ± 174 NTU) and drastically drops as we approached towards the transition zone 'Mahuva' (34.7 ± 22.8 NTU) and Open Sea sites (<10NTU) respectively (Table 1). Seasonal fluctuations of salinity and turbidity are found to be only significant inside the Gulf sites, where salinity drops and turbidity elevates during monsoon because of heavy rainfall, which exert a dilution effect as well as increases the re-suspension rate of benthic sediments. At the sea-mouth and open sea dilution effect by seasonal downpour is negligible as well as water-circulation doesn't put any significant impact on turbidity level due to the rocky sea bed.



During the study period, Dissolved Oxygen (DO) concentrations in all the five study sites varied between 4.37 mg.L^{-1} and 7.0 mg.L^{-1} (Table 1). Maximum DO was recorded at the 'Veraval' site and it gradually decreases as seawater flows towards the inland. Depletion of oxygen level in inland sites viz. 'Koliyak' and 'Alang' might be influenced by various factors involving bio-geochemical processes, microbial decomposition and anthropogenic interventions. Inside the Gulf area turbidity increases rapidly, which has an inverse relation with DO concentration (Fig. 3). High levels of turbidity are limiting primary productivity by restricting sunlight penetration, which results in low dissolved oxygen production in the Gulf water. In addition to this, excessive loads of nutrients and organic debris discharged by the riverine estuaries enhance seasonal plankton bloom inside the Gulf, facilitating the eutrophication process that leads to the decline of DO level [18]. The anthropogenic impact has played an extensive role in keeping the turbidity level high and oxygen level low by oil spillage from the ship wreckage industry situated at 'Alang', which releases organic chemicals that require oxygen for decomposition. Simultaneously, organic matter inputs from nearby agricultural fields and untreated disposal of urban runoffs increase the pollution index of Gulf water resulting in an upsurge of oxygen demand. Therefore, hypoxia in the inland sites is the result of water contamination due to sewage disposals, fecal pollution, industrial runoffs and various other types of anthropogenic interventions.

The seasonal peak of DO was recorded only in the winter months because of drop in water temperatures as warm water decreases the solubility of oxygen level (Fig. 2) [32]. Photosynthesis is the primary process affecting the dissolved-oxygen/temperature relation. Water clarity, strength and duration of sunlight, in turn, affect the rate of photosynthesis [33].

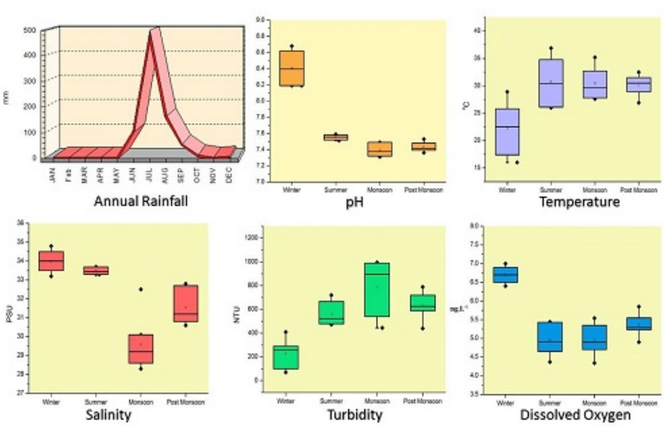


Fig. 2 Seasonal variation of climatic and hydro-chemical parameters inside Gulf zone

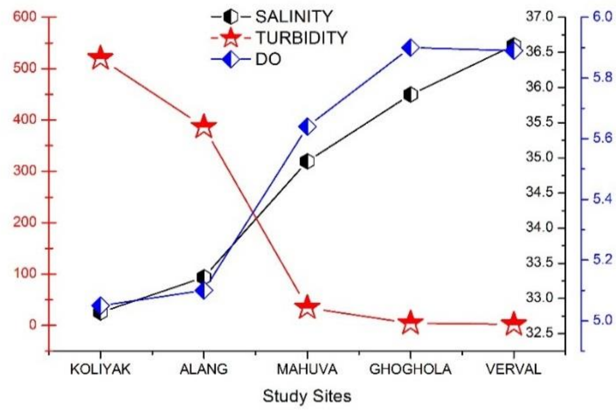


Fig. 3 Site wise variation of hydro-chemical indices

3.2 Dynamics of bacterial enteropathogens across the Gulf setting

Total bacterial count (TBC) varied between 130-37449CFU/mL annually with an increasing trend from the onset of summer and remain high throughout the monsoon and post-monsoon period before dipping in the winter. The seasonal effect is most prominent inside the Gulf where the highest TBC (9528 CFU/mL) could be obtained in post-monsoon with the increase of seawater intrusion. TCC and TEC varied 2-789 CFU/mL and 0-189 CFU/mL respectively throughout the study period (Table 1). Coliforms were frequently detected inside the Gulf, occasionally in the transition zone, and seldom in the open sea. Whereas the existence of *E. coli* could only be detected inside the Gulf, which indicates fecal contamination in the Gulf water. The abundance of both coliforms and *E. coli* increases gradually from winter months and attain its peak in monsoon, and thereafter from the onset post monsoon, the bacterial load shrinks suddenly (Fig. 4). The prevalence of non-halophilic coliforms in the considerably high saline water is a significant finding, which indicates their high adaptive potential and inherent osmoregulatory capability to tolerate environmental stress.

Spatial variation of CVC across the Gulf coast indicates a gradual increase of Vibrio load with the increase of salinity and decrease of turbidity. Though no significant positive or negative correlation has been established amongst CVC with salinity and turbidity, however, CVC decreases drastically below 33 PSU salt concentration and above 80NTU turbidity. The annual range of CVC varied between (24-1003 CFU/mL), (54-1779 CFU/mL) and (80-3115 CFU/mL) inside the Gulf, transition zone and open sea respectively (Table 1). Inside the Gulf, a high abundance of CVC was recorded in summer months influenced by ambient water temperature (avg. 33^{o}C) as well as higher wind pressure and tidal current, which facilitates intrusion of sea water. Thereafter, the Vibrio load reaches its maxima during monsoon season, when continuous splashes of rainwater made the gulf water highly turbid by resuspension of muds of intertidal mudflats and sediments carried by multiple riverine estuaries. Finally, CVC drastically drops in winter due to a decrease in temperature and turbulence in Gulf water. A similar trend of dispositional variation of CVC has also been observed in the transition zone, with a little monsoonal peak due to flooding and sediment resuspension. However, no significant seasonal variation of CVC has been noticed in the open sea (Fig. 4).

A massive abundance of *V. alginolyticus* was obtained in all the study sites throughout all the seasons of GoK. *V. alginolyticus* was found to be the highly dominant Vibrio species in the euhaline habitat of Gulf water. Out of 205 water samples collected in the yearlong sampling, 84.87% of the samples were positive for *V. alginolyticus*, 10.73% for *V. parahaemolyticus* and 2.92% were positive for *V. cholerae*. Earlier studies identified *V. alginolyticus* as an opportunistic pathogen that can tolerate a long-range salinity gradient (0 to >28PSU) and environmental temperatures (13.4-35.6^{0}C) [2, 34]. Here the effect of high salt density (>30 PSU) along with persistently high turbidity (386 ± 172 NTU) and hot weather might be the crucial determinant of the explosive abundance of *V. alginolyticus* in the Gulf. However, *V. cholerae* could be isolated in very few numbers from the GoK and their distribution is strictly restricted inside the high turbid water of GoK. *V. cholerae* is autochthonous in the riverine-estuarine habitat of low to moderate salinity (<20PSU) and here its existence in the high saline Gulf water (28.3-34.8 PSU) signifies their high adaptive potential and tolerance level against environmental extremities, which succour them to establish a wide variety of habitat choice.

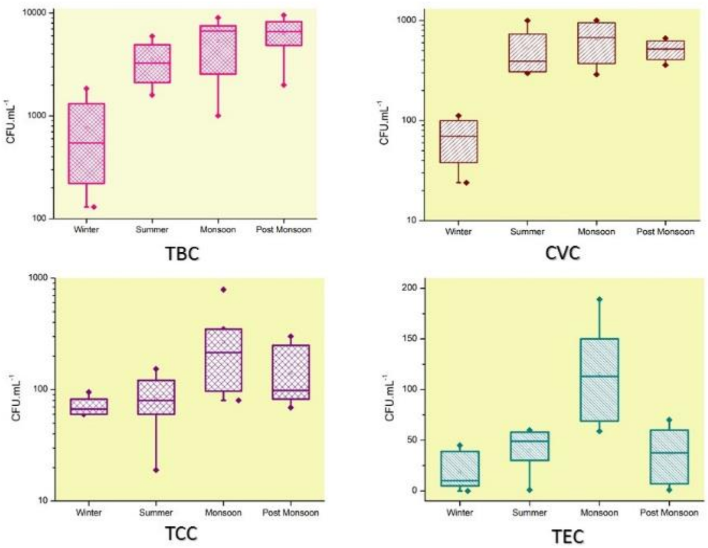


Fig 4 Seasonal variation of bacteriological entities inside the Gulf zone

3.3 Correlation between environmental predictors and bacteriological entities

The interplay of environmental indices regulating the enterobacterial dynamics in the high saline Gulf ecosystem has been assessed by applying Pearson's correlation technique. . During the yearlong expedition, it has been observed that the seasonal abundance of the Gulf enterobacterial community has a complex relationship with the hydrological properties of that marine environment. The statistical analysis exhibits that all of the hydrological parameters are positively correlated with the bacteriological load (TBC), however no contributing impact of temperature could be obtained on the dynamics of TBC, except at deep inside the Gulf ('Koliyak') where TBC increases with the increase of seasonal temperatures ($r=0.37$; $p>0.05$).

The highest load of coliforms could be obtained from the study sites located inside the Gulf area, where turbidity is exceptionally high and human exposure to marine water is abundant. Abundance of free-floating coliforms (TCC) and E. coli (TEC) showed a positive and significant correlation with turbidity (r= 0.62; p<0.001 and r= 0.68; p<0.001 respectively), whereas negatively linked with salinity quotient (r= -0.35; p<0.05 and r= -0.39; p<0.05 respectively). A strong association between TEC and turbidity indicates the bentho-pelagic coupling capacity of E. coli [35]. Although, the inverse relationship with salinity is a usual outcome in the case of halophobic coliforms, the prevalence of *E. coli* in such salty water of above 30PSU salinity is quite unusual. They must have adapted several osmoregulatory systems which synthesizes specific osmoprotectant molecules to overcome the tremendous osmotic upshock in the marine environment [36]. Increased alkalinity of seawater is another detrimental factor for coliforms. A weak negative correlation was observed between TCC and pH, however, pH ≥8 contributes to the deleterious effects on coliform survival. Both TCC and TEC dynamics are independent of temperature and DO fluctuation in marine water.

The vibrio load (CVC) of GoK was strongly associated with the water salinity (r=0.441; p<0.1), which substantiates the earlier theory of salinity-dependent Vibrio abundance, established by both *in situ* and *in vitro* studies [2]. DO was found to be another contributing factor of CVC in the Gulf ecosystem. Elevation of oxygen level significantly increases Vibrio load in the surface water (r= 0.68; p<0.001).

The oxygen density exhibits a strong negative correlation with turbidity i.e dissolved particulates load in the water (r= -0.53; p<0.05). The poor oxygen capacity in the high turbid Gulf is due to the congregation of zooplanktons, sestons, and other floating microorganisms which consume more oxygen molecules. Therefore, highest free floating Vibrio load is obtained in the well-oxygenated waters of open-sea sites and eventually turbidity shows a negative correlation with CVC (r= -0.33; p<0.1). Interestingly, the high load of suspended solids with poor oxygen levels is the preferable niche choice amongst the enteropathogenic Vibrio species of the Gulf ecosystem. However, this is contradictory with the riverine Vibrios of Ganges Delta, where turbidity is positively associated with CVC [24, 27]. A weak correlation could be obtained between temperature and CVC (r=0.28; p<0.1). High temperature is favorable for Vibrio growth, however optimum growth of this enterobacteria occurs in between 30 and 35°C and in a range of pH 7.6-8.6 [37].

The correlation analysis of pathogenic Vibrio species with the hydrological parameters provides deep insight into the dynamics of enigmatic diarrheal pathogens in response to environmental stimulants. The most abundant Vibrio of GoK i.e. V. alginolyticus shows a highly significant negative correlation with DO level (r= -0.65; p<0.001), which signifies its strong affinity towards hypoxic water of the Gulf, which might have been polluted by sewerage, agricultural and industrial disposals. It also shows affinity towards temperature and moderate alkaline water, which exhibits a positive association with temperature (r=0.45; p<0.1) and inverse relation with water pH level (r=0.43; p<0.05). Interestingly it has no correlation with salinity, which indicates an independent distribution of V. alginolyticus in any given salt density of Gulf water.

However, the V. parahaemolyticus load is positively correlated with salinity level (r=0.37; p<0.05) and negatively linked with turbidity quotient (r= -0.53; p<0.001). Both V. parahaemolyticus and V. alginolyticus flourish with the increase of temperature and decrease of alkalinity up to the threshold limit. Extremely high or extremely low temperature, as well as pH, are both detrimental to these environmental pathogens (Table 2).

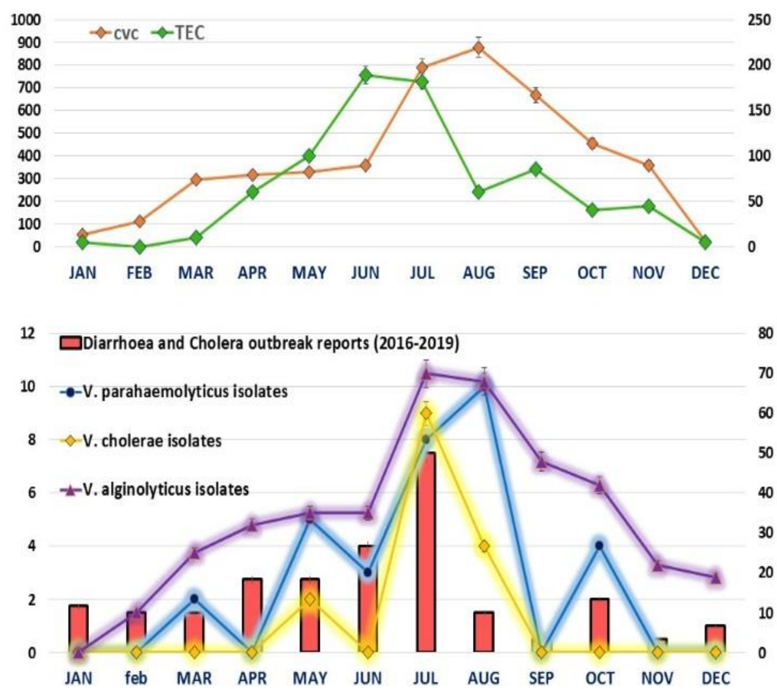
The high saline habitat of seawater is usually not a preferable niche of V. cholerae, which is ubiquitous in brackish water. Earlier studies conducted in various aquatic environments observed that the detection rate of *V. cholerae* drastically diminishes above 6.6PSU salinity [2, 38], however, their abundance in the present study in extremely high saline habitat (>33.5PSU) might be facilitated by some hydro-chemical attributes of the Gulf setting. A strong correlation has been observed between V. cholerae abundance and turbidity of water (r=0.75; p<0.001), and the distribution of this pathogen is restricted only in the turbid zone (>250NTU) of GoK. An increase in turbidity results in higher nutrient supply along with the bloom of plankton biomass and elevates the overall productivity of Gulf water [24], which provides more attachment sites for *V. cholerae* to withheld the environmental stressors in a nutrient-rich condition. As it is discussed earlier that turbid water compromises oxygen level, this organism adapted to flourish in less-oxygenated water aided by its anaerobic respiration potential and shows a negative correlation with DO concentration of water ($r=-0.34$; $p<0.1$). However, temperature fluctuation and pH don't exert any significant impact on this high saline V. cholerae, but salinity shows a significant negative correlation ($r= -0.87$; $p<0.001$), as anticipated.

3.4 Seasonality of enteropathogens and diarrheal disease burden

In our endeavor to unfold the long-standing paradox of the environmental process by which the diarrhoea endemicity is sustained in the Gujarat plain, we attempted to understand the mechanism that the Gulf water can play as a potential reservoir of diarrheal pathogens during epidemics. To our existing knowledge, this is the first longitudinal comprehensive study report in the seasonal dynamics of hydro-chemical variants of the Gulf aquatic regime, addressing the dynamicity of its enterobacterial inhabitants and simultaneously correlating their abundance with environmental factors in relation to diarrhoea and cholera paradigm of this zone.

The yearlong comprehensive study reveals that the dynamics of environmental and aquatic attributes contributed to the seasonal abundance and distribution of clinically important enterobacterial species in Gulf water. Seasonal precipitation rate shows a sharp spike in July (Fig. 2), when the abundance of *E. coli, V. alginolyticus* and *V. cholerae* was were also at its peak (Fig. 5). However, *V. parahaemolyticus* flourishes just after the rainfall event in the month of August and its second peak was noticed at the post-monsoon period when salinity level rises in surface water (Fig. 5). As we have discussed earlier, the seasonal abundance of *V. alginolyticus* is independent of salt concentration, a single peak of its abundance could be noticed in monsoon when turbidity level reaches its maxima. Whereas two seasonal peaks of *V. cholerae* are attributed to the effect of temperature in summer and turbidity in monsoon (Fig. 5). Thus, it is evident from the present study that the aquatic settings of GoK and its hydro-chemical quality of water induces the seasonal peaks of environmental strains of enteropathogenic bacteria. Interestingly, the enterobacterial dynamics also coincides with the pattern of diarrheal disease outbreaks in the community, as evident from the IDSP diseases surveillance data (IDSP 2021).

Fig. 5 Seasonal data of enteropathogenic bacteria on the diseases data of the study foci. Disease data collected from IDSP website. https://idsp.nic.in/index4.php?lang=1&level=0&linkid=406&lid=3689



Exploration of disease surveillance data have very interestingly depicted a marked seasonality in the numbers of outbreak reports attributed to enteropathogenic Vibrio species. A single distinct peak of diarrheal incidences could be observed in monsoon (July) and could be nicely extrapolated on the seasonal abundance data of *V. cholerae*, *V. alginolyticus*, *V. parahemolyticus* and *E. coli*, which signifies the direct consequential impact of environmental enteropathogens on the community health status (Fig. 5). Interestingly, a deviation in diarrheal diseases pattern in the present study could be observed from our earlier investigations carried out in the century-old diarrhoea endemic realm of the Indian Ganges delta, where two seasonal peaks (summer and monsoon) of diarrheal incidences have been noticed [26]. This is explained by the prolonged drought and very low precipitation rate in the summer months, which could have a strong impact on the seasonal prevalence of enteropathogens and subsequent disease transmission profile of the Gulf area.

4 Conclusion

The present work evidentially establishes a fact that the Gulf of Khambat, India and its adjoining coastal Arabian Sea zone is the potential reservoir of various enteropathogenic bacteria with its hydro-chemical quality of water indicating strong pollution by sewage discharge, faecal contamination and industrial runoffs. Simultaneously, the seasonality of climatic and hydrological factors and its impact on the dynamics of enterobacterial entities have been reported for the first time from this study foci. Results show that annual variation of salinity at all the study sites is negligible in comparison to the seasonal fluctuation of turbidity that indicates the significant impact of turbidity on all the bacteriological entities. High temperature, high salinity, turbidity and sufficient oxygen level is found to be the crucial determinant of Vibrio load. Prevalence of the enteropathogenic Vibrio species viz. *V. alginolyticus, V. cholerae* and *E. coli* could be observed in the most turbid phase of Gulf water, whereas *V. parahaemolyticus* prefers clean water. Coliforms and *E. coli* has been adapted to settle in the high saline Gulf by the help of high turbidity, moderate temperature and optimal pH.

The finding of the present study adds a new arena of the knowledge base about the 'high saline-high turbid' reservoir of enteropathogenic bacteria and its seasonal prevalence regulated by the dynamic aquatic setting of a Gulf zone (GoK in the present finding). This is the first documentation of seasonal dynamics of enteropathogenic bacteria in any Gulf ecosystem of South East Asia. A few studies have been conducted to unfold bacterial distribution associated with coral reefs across the Gulf of Mannar and Gulf of Kutch, however, the enterobacterial distribution in relation to human health impact is hitherto unknown in any Gulf settings of South East Asia. Earlier studies were focused on the toxigenic Vibrios of the Gulf of Mexico, exploring the understanding of aquatic factors linked with bacterial dynamics. However, the aquatic setting of the Indian Gulf is different, and its impact on the array of enterobacterial entities along with associated health impact on the adjacent coastal population is an exclusive report from the present study. Simultaneously, the impact of the study is significant in the public health perspective of the coastal community. Human exposure to the Gulf water is frequent across the Gulf coast due to various socio-recreational purposes including bathing, performing religious rituals and household chores. Additionally, the huge number of workers of the ship wreckage industry at the adjacent 'Alang' site are regularly exposed to seawater. Furthermore, gulf shores are an important seafood harvesting zone, where contaminated water poses a potential threat to edible fishes and crustaceans, and also increases consumer health risk. Therefore, future long term systematic studies are advocated to understand and predict enteric pathogen dynamics through models relating to diarrheal disease incidence patterns. Enteropathogenic Vibrios and coliforms in association with climatic and hydro-chemical factors viz. precipitation rate, water temperature, salinity, turbidity and oxygen level, are the potential cluster of indicators collating an effective 'bio-monitoring tool' for the climate-sensitive enteropathogen dynamics of the coastal ecosystem. This will help to develop a better 'composite index tool' (for an early warning system) to predict future outbreaks, especially in such a vulnerable coastal population. Simultaneously adaptive efforts should be focused on the appropriate treatment of sewage and industrial runoffs as well as development and proper management of irrigation work, for improving the water quality and reducing pathogenic contamination. Since all the hydro-chemical and biological markers exceed the threshold level in marine water of the study zone, access to Gulf water should be restricted in all practical purposes for anthropogenic activities.

Global environmental change is likely to increase the magnitude and frequency of extreme climate events such as floods, droughts and coastal storms and also increase in sea level and water temperature, which alters the biogeochemical processes in aquatic systems. The Gujarat coast of India is especially vulnerable to periodic cyclonic storms and other natural disasters, which are thought to play a profound role in diarrheal disease dynamics. The present study established a link between heavy rainfall and temperature with subsequent diarrheal pathogen dynamics and disease outbreak pattern. This is important to develop a long-term monitoring program to achieve the resilience of the Gulf ecosystem from the adverse effect of climate change and improving water quality to an optimal range to neutralize potentially adverse future health impacts.

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	Koliyak	Alang	Mahuva	Ghoghola	Veraval
pH	7.55-7.56	7.51-7.73	7.71-7.75	7.68-8.44	7.58-8.44
Temp (^{o}C)	27.4-35.5	26.4-36.4	25.8-35.8	28.3-36.7	26.7-36.4
DO (mg.L^{-1})	4.37-6.9	4.67-6.8	5.55-6.7	5.87-6.9	5.77-7.0
Turbidity (NTU)	306-995	70.5-772	40.1-140.5	9.6-10.12	3.6-3.68
Salinity (PSU)	28.3-33.7	30.5-34.8	35.7-35.9	36.6-36.7	36.0-36.1
CVC (CFU.mL^{-1})	24-1003	28-998	54-1779	98-2367	80-3115
TEC(CFU.mL^{-1})	10-156	56-189	0-6	0	0
TCC(CFU.mL^{-1})	112-667	333-789	12-234	4-19	7-23
TBC (CFU.mL^{-1})	130-3900	5450-5999	3900-11950	9500-11100	11256-37449

Table 1 Site wise variation of hydro-chemical and bacteriological attributes

	pH	Temperat ure	Salinity	Turbidity	Dissolved Oxygen
TBC	+	NC	+	+	+
TCC	-	NC	--*	++**	NC
TEC	NC	NC	--*	++**	NC
CVC	NC	++	++	--	++*
V. alginolyticus	--*	++	NC	+	--**
V. parahaemolyticus	--*	++*	++*	--	--
V. cholerae	NC	NC	--**	++**	--

Table 2 Correlation matrix depicting relationship between hydro-chemical variants and bacteriological attributes
'NC' indicates No Correlation; '*' Indicates significant at 0.05 level;
'**' Indicates significant at 0.001 level