

Preponderance of Enteric Pathogens Along the Coastal Waters of Southern Kerala, Southwest Coast of India

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Abstract Bacterial population in the coastal waters of southern Kerala was investigated at six selected transects. From the present study six major groups of enteric bacteria, viz, *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Vibrio parahaemolyticus* and *Vibrio cholerae* are screened. The distribution of enteric bacteria was more in the nearshore than the offshore environments and the highest occurrence was found along the Cochin transect followed by Neendakara attributed to indiscriminate discharge from the industrial effluents, harbour and municipal sewage enriched with enteric microbes. The minimum occurrence was encountered at Veli transect might be due to the acidic effluent discharged from Travancore Titanium Products Limited (TTP), seems to have severe drop in seawater pH and adversely affected proliferation of enteric pathogens. Relatively low bacterial load in the offshore stations compared to nearshore may be attributed to salinity variations and marginal stress from the saline water. The result of the study provides insight into the ecology of this aquatic species and is potentially important for the understanding of the epidemiology of enteric diseases on a regional scale.

Keywords Southwest Coast Of India, Coastal Waters, Enteric Bacteria, Faecal Coliforms, *Escherichia Coli*, *Vibrio Cholerae*

1. Introduction

Innovative research relating oceans and human health is advancing our understanding of disease-causing organisms in coastal ecosystems. Novel techniques are elucidating the loading, transport and fate of pathogens in coastal ecosystems, and identifying sources of contamination. This research is facilitating improved risk assessments for seafood consumers and those who use the oceans for recreation. Marine ecosystem is being threatened by the discharge of untreated sewage wastes and industrial effluents which ultimately affects the sustainability of living resources and public health. These wastes carry enormous level of microbial pathogens to the marine environment and results in negative impact on the marine resources thus causing economic loss[1]. Some microbial pathogens in the coastal environment are indigenous to the oceans, including *Vibrios*. Whereas others like *Escherichia coli*, *Salmonella* spp. and *Shigella* spp. are allochthonous which introduced through agricultural, urban surface runoff, waste water discharges and from domestic and wild animals. Most of the *Vibrios* and *Salmonella* spp. are pathogenic to humans and some have fatal infections[2-5]. Infections with *Vibrios* are known to be associated with either consumption of seafood or exposure to

marine environment[6]. The presence of faecal coliforms forms representative for the assessment of coastal recreational water quality. The present investigation highlights the occurrence and distribution pattern of enteric pathogens in marine water. It also evaluates the influence of anthropogenic inputs and raw sewage on the incidence of these bacteria at inshore and offshore region along Kerala coast.

2. Materials and Methods

Six transects were established for the study along southern Kerala coast between latitudes 9° 57' N and 8° 29' N and longitudes 76° 14' E and 76° 53' E stretch from north to south. The transect I (Cochin), transect II (Alleppey), transect III (Kayamkulam), transect IV (Neendakara), transect V (Paravur), and transect VI (Veli) respectively stretches from north to south (Figure 1). Four stations, on each of these transects were selected for sampling viz, nearshore, 2 km, 5km, 10km offshore across the coast. The water samples were collected during the cruise programme of *CRV Sugar Purvi*, from 7th Jan to 10 Jan 2004. Duplicate sampling and analysis were performed for each station. *In situ* temperature was recorded using a thermometer (1–51°C range within ± 0.1°C; Brannan, UK). The samples for dissolved oxygen (DO) were fixed onboard and the remaining water samples were collected in 2 litre PVC bottles kept in the ice boxes and brought to the laboratory at the earliest. DO was estimated according to Winkler's method[7]. Salinity was determined using a Digi

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Auto Salinometer (Model TSK, accuracy ± 0.001) and the pH using an ELICO LI 610 pH meter (accuracy ± 0.01). Six major groups of enteric bacteria were selected and the selective media used for the growth of bacteria were Membrane Filter Coliform (MFC) agar for faecal coliforms, M-7hr FC Agar for *Escherichia coli*, Xylose Lysine Deoxycholate Agar for *Shigella* spp. and *Salmonella* spp. and Thiosulphate Citrate Bile Sucrose Agar for the isolation of *Vibrio parahaemolyticus* and *Vibrio cholerae*. The spread plate technique was adopted to enumerate the enteric bacteria except *Escherichia coli* using 0.1 to 0.5 ml of the sample and the results were reported as Colony Forming Units (CFU/ml). Membrane filter technique was used for the isolation of *Escherichia coli* using 10 ml sample.

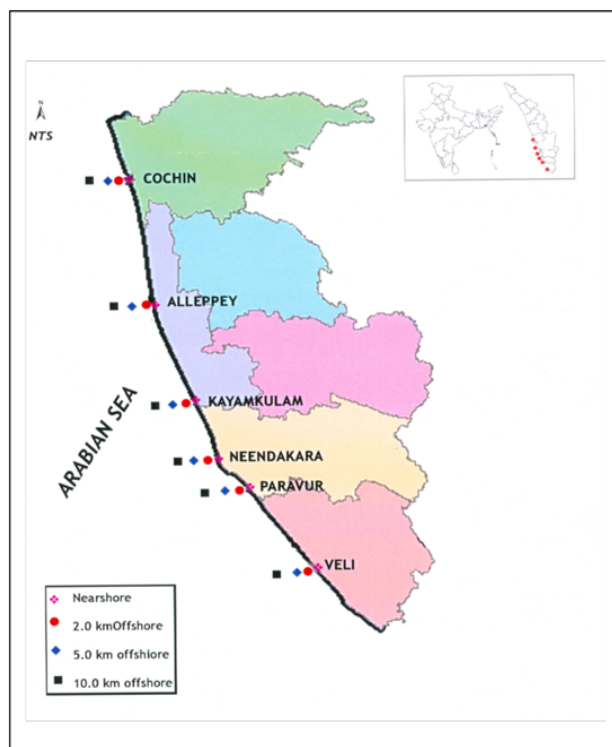


Figure 1. Study area with sampling sites

3. Results

3.1. Hydrographic Conditions

Coastal waters of Kerala had a warm humid climate. Among stations, atmospheric temperature varied from 28.4°C at Paravurnearshore (estuary) to 31.6°C at Alleppey 10 km offshore. The surface water temperature ranged from 26.0°C (Paravur 2 km) to 28.3°C at Alleppey 5 km offshore and Velinearshore. The variation in surface water temperature in all station in different transect might be due to intensity of solar radiation, evaporation and fresh water in flow. Further statistical analysis showed a positive correlation ($r = 0.66$) between air and water temperature. In the present study Velinearshorewater recording the maximum temperature may be due to acidic effluent discharge from Travancore Titanium Product (TTP) factory. This observation is in

agreement with that of the study conducted by Ouseph[8]. There was significant fluctuation in salinity among the stations. The maximum salinity of 34.6 was recorded at Veli, 10 km offshore and the minimum (14.7) was recorded at Cochin nearshore (estuary) Figure 2. Salinity results showed positive correlation with temperature ($r = 0.68$). Among the stations, estuarine stations recorded low salinity compared to offshore stations. This might be due to large quantity of fresh water discharge from backwaters. It is one of the prime factors, which influences the abundance and distribution of the fauna and flora in the estuarine and coastal waters. Salinity is also known to affect the distribution of calanoid copepods in estuaries[9]. Among the stations, Velinearshore recorded the lowest pH (3.6) when the highest (8.23) was recorded from Cochin estuary Figure 3. The pH values showed great fluctuation among the stations. The present study recorded an acidic pH of 3.60 at effluent discharge point of Velinearshore indicating the extremity of acidic factory effluents discharged from Travancore Titanium Product Factory (TTP). Similar observations were also made by Robin et al. and Prigilal[10 and 11]. Abdul Azis and Nair[12] revealed that, on a full production day total quantity of H_2SO_4 discharged from TTP plant consist of 32.8 tons. Nearshore stations recorded slightly low pH compared to offshore stations. The lower pH at nearshore stations may be due to freshwater influx, dilution of sea water, low temperature and decomposition of organic matter as suggested by Ganesan et al.[13] and also due to industrial discharge. There was not much variation in DO concentration among the stations. The maximum concentration (5.85 mgL^{-1}) was recorded at Alleppey 5 km offshore and minimum (3.28 mg L^{-1}) was recorded at Velinearshore.

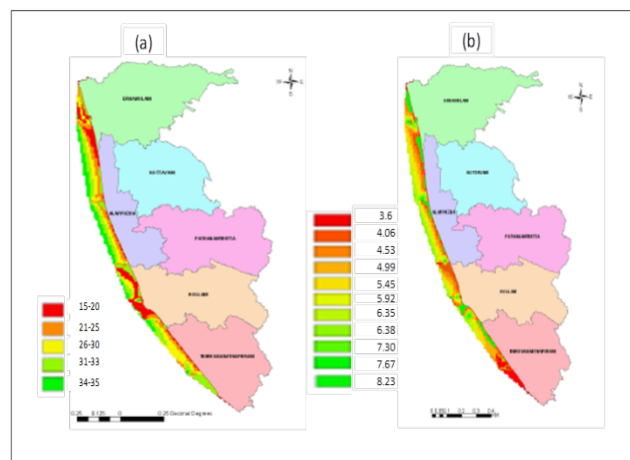


Figure 2. Variation of (a) salinity and (b) pH along the southern Kerala coast

3.2. Total Viable Count (TVC)

Total viable count in water samples fluctuated broadly from 285 CFU/ml at Velinearshore to 36.43×10^3 CFU/ml at Cochin estuary. Nearshore waters were recorded much higher than in the offshore water might be due to decomposition of organic matter, thereby increasing the bacterial population Figure 3.

3.3. Faecal Coliforms (*Escherichia coli*)

Escherichia coli were also predominantly seen from estuarine samples. Faecal coliforms ranged from nil at many of the offshore stations to 480 CFU/ml. The maximum population was observed in the nearshore surficial water at Neendakara. Distributions of *Escherichia coli* at different stations were illustrated in Figure 4.

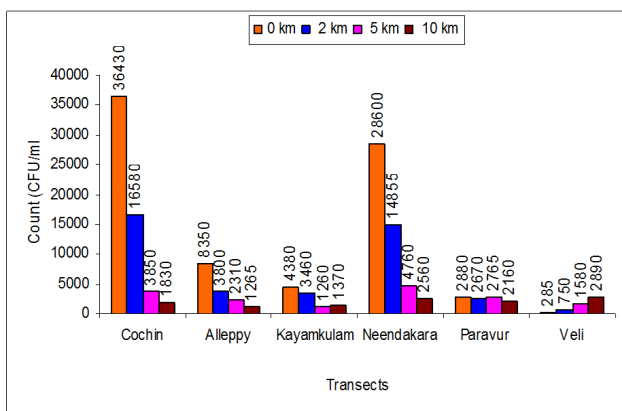


Figure 3. Variation of TVC (CFU/ml) along the transects

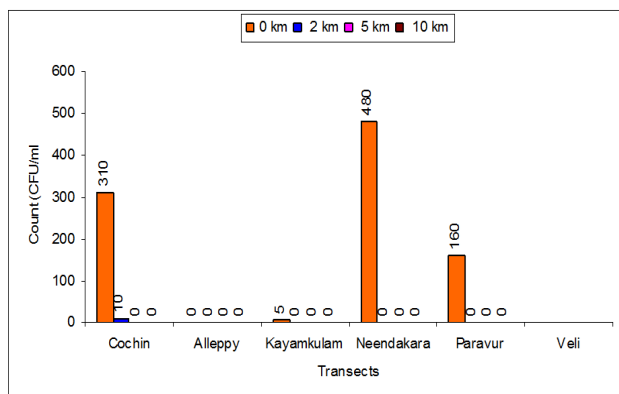


Figure 4. Variation of Faecal coliforms (CFU/ml) along the transects

3.2. *Shigella* spp.

Cochin nearshore (estuary) recorded the maximum count of *Shigella* spp. (3860 CFU/ml) and their presence confined mostly to the estuarine and nearshore waters of all transects. Whereas Cochin 5 and 10 km, Alleppey 2, 5 and 10 km, Kayamkulam 2, 5 and 10 km, Neendakara 5 and 10 km, Paravur 10 km and Velin earshore did not record *Shigella* spp. Distributions of *Shigella* spp. at different stations are shown in Figure 5.

3.3. *Salmonella* spp.

Among stations count of *Salmonella* spp. was very low except Neendakara nearshore (estuary) where it recorded the maximum count of 670 CFU/ml. Distributions of *Salmonella* spp. at different stations are shown in Figure 6.

3.4. *Vibrio parahaemolyticus*

Alleppey nearshore recorded maximum density of *Vibrio parahaemolyticus* (1030 CFU/ml), while they were not

enumerated from samples at stations like Neendakara 10 km and Veli near shore. Presence of *Vibrio parahaemolyticus* was seen up to 10 km offshore. Distribution of *Vibrio parahaemolyticus* at different stations shown in Figure 7.

3.5. *Vibrio cholerae*

Vibrio cholerae were prevalent in the nearshore to offshore regions of most of the transects through estuaries showed their maximum presence. Maximum densities of 1670 CFU/ml were enumerated at Cochin nearshore (estuary) whereas at Neendakara 10 km and Veli nearshore, they were not enumerated. Distributions of *Vibrio cholerae* at different stations were shown in Figure 8.

4. Discussion

Microbiological examination of coastal water has a special status in marine pollution studies, as it is a direct measurement of deleterious effect of coastal pollution on human health through food chain. It is essential to monitor marine microbial population to ensure the safety of seawater for recreational purpose. Microorganisms in seawater include several harmful bacteria which are capable of causing diseases such as diarrhea and cholera and make potential threat to human health [14 and 15]. These pathogenic organisms present in coastal waters contaminated by domestic sewage and other organic waste materials.

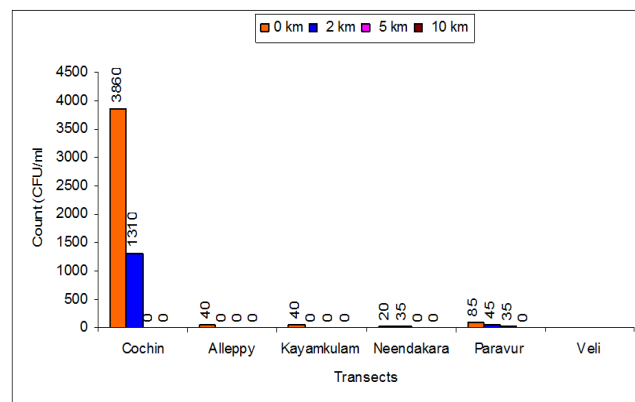


Figure 5. Variation of *Shigella* spp. (CFU/ml) along the transects

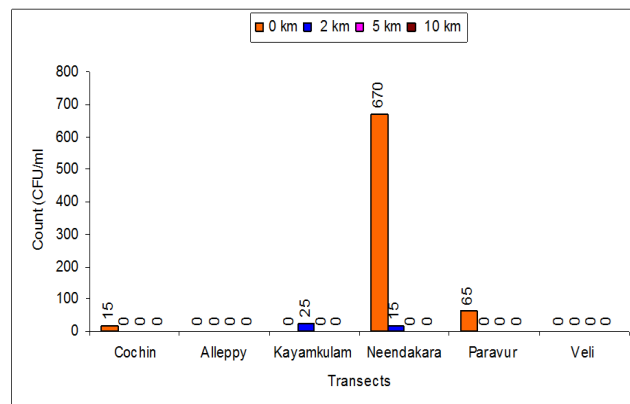


Figure 6. Variation of *Salmonella* spp. (CFU/ml) along the transects

The trend in the distribution of heterotrophic bacterial population in different transects showed relatively high density of TVC except at Veli transect, where effect of acidic effluent might have considerably reduce the total viable count. Maximum TVC density of 36.4×10^4 was recorded at Cochin nearshore(estuary). This result is in agreement with the works of Ramaiha and Chandramohan[16]. Generally coliforms occur in large number in coastal water, which is faecally contaminated. Their high incidence can be accompanied by the pathogenic bacteria like enteropathogenic *Escherichia coli*, which occur in very small numbers in faecal matter. Therefore, total coliforms are generally considered as indicators of water pollution. Beyond 2 km offshore coliforms were not generally enumerated. At Neendakaraneareshore(estuary) recorded maximum density faecal coliforms of 460 CFU/ml were observed.

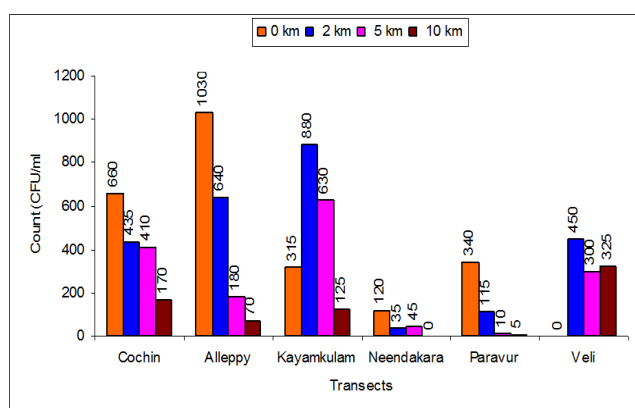


Figure 7. Variation of *Vibrio parahaemolyticus* (CFU/ml) along the transects

The overall occurrence of enteric bacteria in water and sediment shows that the most dominant group envisaged be the *Shigella* spp. followed by *Vibrio cholerae*, *Vibrio parahaemolyticus* and the least one for *Salmonella* spp. *Vibrio* spp. are widely distributed in marine environment and studied extensively by various authors[17 and 18] *Salmonella* spp. like organisms, of which many are pathogenic and survive very short in coastal water. The maximum densities of 3860 CFU/ml *Shigella* spp. were enumerated at Cochin near-shore(estuary). The present result is comparable to the earlier reports of Sarthre et al.[19]. *Vibrio cholerae* is a pathogenic organism that causes the dreaded cholera. Existence and the distribution of Vibrios in coastal waters of southern Kerala coast is mainly from polluted water and waste water effluents and their density was found more at near the water surface of polluted area and their habitat decreased from the source of pollutants of the site of waste water discharge. Many species of Vibrios are natural inhabitants of seawater; prefer to survive in saline pH. All stations except Velinear-shore (EDP) and Neendakara 10 km offshore waters have enumerated *Vibrio cholerae*. The incidence of cholera in the coastal belt of southwest cost the country is frequent[20]. Alleppey 10 km offshore accounted high density of *Vibrio parahaemolyticus* like organisms (1030 CFU/ml). *Vibrio parahaemolyticus* is frequent in all station except Velinear-

shore(EDP). As seawater is a natural habitat for *Vibrio parahaemolyticus*, their high number is quite expected. Pradeep et al.[21] has reported salinity was found to be the major limiting factor for the distribution of *Vibrio parahaemolyticus*. High salinity of seawater helps the survival and growth of *Vibrio parahaemolyticus*. Lokabharathi et al.Davieset al.[22] has reported that zooplankton can enrich *Vibrio parahaemolyticus* from their ambient environment. The present investigations are comparable to early report of Sanjeev and Mahadeva[23]. *Salmonella* spp. in water was very low and was not enumerated from most of the stations whereas Neendakaraneareshore(estuary) recorded the relatively high numbers *Salmonella* spp. regarded as an index of pollution of coastal waters. Studies showthat their incidence even in low numbers in marine water is of human or animal origin[24]. The variation microbial population coastal waters may also be attributed to the different rates of growth and survival of these pathogens in the marine habitat. Relatively low bacterial load in the offshore stations compared to nearshore may be attributed to salinity variations and marginal stress from the saline water.

The occurrence of enteric bacteria in water sample showed the maximum percentage was in nearshore sample of all groups. This enhanced levels in the coastal waters might be due to re-suspension of microbes in the surf zone along with high level of organic matter present in the sediment may also be a reason for promoting the survival of enteric pathogens especially Vibrios.Dale[25] and Davies et al.[26] pointed out that the interaction of microorganisms with sediments may enhance their survival by reducing exposure to stressors such as infrared radiation and predation or by increasing the availability of nutrients. Van Donsel&Geldreich[27] and Goyal et al.[28] noticed that sediments appear to provide the most conducive ecological niche for the survival of the pathogens. The percentage occurrence of faecal coliforms, *Escherichia coli*,*Shigella* spp. and *Salmonella* spp. was found to be more in the nearshorewaters than offshore. The presence of faecal coliforms in the coastal waters indicates that the faecal contamination is from the human and animal source.

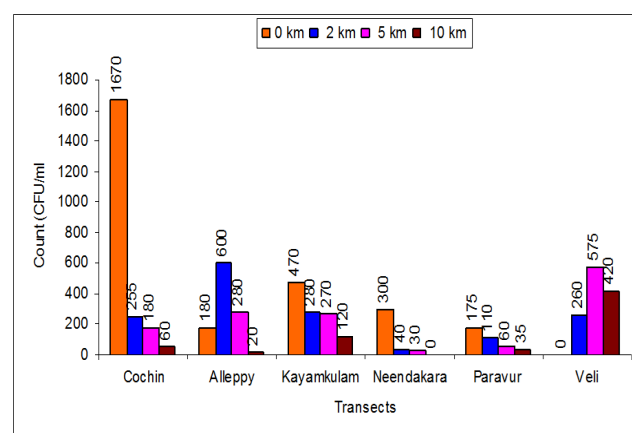


Figure 8. Variation of *Vibrio cholerae* (CFU/ml) along the transects

The station wise occurrence of enteric bacteria indicates

that Cochin transect was found to be highest population followed by Neendakara and percentage occurrence of all groups especially at nearshore waters. The river discharges which have been attributed as stimulants for higher occurrence of microbial population in the coastal waters[29]. The coastal waters of Cochin transect receives huge fresh water from river Periyar and large quantities of waste water from cities and variety of industrial effluents, harbour and municipal sewage water from greater Cochin area with minimal treatment which carries enormous level of pathogens. Movement of ships, barges, fishing boats, extensive dredging, and oil tanker activities were also among the major sources responsible for deterioration of water quality of Cochin transect. The occurrence of *Salmonella* spp. in Neendakaratract and *Shigella* spp. in Cochin transect was probably due to the discharge of polluted waters from adjacent land or coastal area. Pradeep and Lakshmanaperumalsamy[30] noticed the main source of faecal coliforms and other enteric pathogens in Cochin transect is through river water, which carries land runoff from urban and rural areas, sewage discharges and storm water. The present observation corroborate with the above findings.

5. Conclusions

The results of the present study reveal that the coastal waters of Kerala being a dumping ground of the untreated sewage wastes and industrial effluents enhances the microbial load especially at nearshore water. The occurrence of *Vibrios*, *Salmonella* spp. and *Shigella* spp. in marine ecosystem is alarming, even though their count was low, it exceeds from the legal limit. Study recapitulated that our coastal waters are stock up with autochthonous *Vibrio cholerae* and make an ample chance for disease outbreak. The coastal contamination due to enteric bacteria leads to quality deterioration of marine resources that pose a human health hazard and subsequent economic loss. It can be concluded that Cochin is the contaminated site characterized by high rate of enteric pathogens.

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