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ASSESSMENT OF POLLUTION INDICATORS AND ANTIBIOTIC RESISTANT STRAINS FROM WATER AND SEDIMENT SAMPLES OF NAGAPPATINAM COAST

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ABSTRACT

Coastal areas are complex formations including bays, estuaries, beaches and large semi-closed areas, which are highly valued as recreational resources and immensely polluted by several sources. Fecal contamination of marine environment from urban development and animals forming is increasing worldwide at an alarming rate. The emergence of antibiotic resistant bacteria is predictable in any environment where antibiotics are released. The transfer of resistant bacteria to humans could occur via water and food if plants are watered with surface waters or sewage sludge, if manure is used as a fertilizer and etc. In this study, the water and sediment samples were collected from Nagappatinam (10.25º and 11.40º North Longitude and 76º 49º and 80.01º East longitude) beach region during July 2014 – August 2014 for pollution indicator and antibiotic resistant analysis. A total of 120 strains (60 *Vibrio* sp. and *E.coli*) were selected and challenged against 10 different standard antibiotics for antibiotic resistant study. In this study, total viable counts (TVC) were in the order of enormity above 10^3 CFU/mL or CFU/g for both the samples (water and sediment) during July and August months. In July water samples, mean counts of TVC, TC, TS, TV, FC and VC were 101000 CFU/mL, 8600 CFU/mL, 980 CFU/mL, 1430 CFU/mL, 190 CFU/mL and 1170 CFU/mL, respectively. The pathogens level was very high in August month compared to July month. The seasonal distribution pattern of different pathogenic bacteria were listed namely, TVC > TC > TV> TS > VC > FC/EC. The overall bacteriological characteristics observed for coastal samples were not good. The 23.3 and 26.6 % of *Vibrio* were resistant to one antibiotic in water and sediment samples, respectively. None of the *Vibrio* colonies from water sample of this site is resistant to 7-10 antibiotics while none of the colonies from sediment sample of this site is resistant to 8-10 antibiotics. The minimal percentage of *E.coli* organisms showed resistance to 5–10 antibiotics. The susceptible strains rates were high in both the samples. The highest frequency of susceptible was exhibited in *Vibrio* sp strains than *E.coli* strains. Hence, throughout impoundment is needed for maintaining the sanitation.

INTRODUCTION

Coastal areas are complex formations including bays, estuaries, beaches and large semi-closed areas, which are highly valued as recreational resources and are widely utilized for a range of activities including bathing, sailing, boating, various forms of surfing and water diving (Mohammed, 2005). The chemistry and biology of coastal waters are very vulnerable to additions of biodegradable and stable compound from land. The relation between population increases in coastal regions and changes in the environment has been known since many years. The Government of India, in the Ministry of Earth Sciences (formerly Ministry of Ocean Development) has been monitoring the levels of marine pollution at about 80 locations along the coastline of the country and this programme is called Coastal Ocean Management and Prediction System (COMAPS) (Vignesh et al., 2014). More and more natural area is converted to urban and suburban development, which has major effects on water quality, availability and movement. Increases in coastal tourism can also bring about water quality problems and waste water is considered as an important source of contamination in coastal areas. These areas are especially significant because two-thirds of the world’s population and 60% of the big cities are located along the coast (Kocasoy, 1989). About 38% of the world's population lives within 100 km of the coast (Small and Cohen, 2004).

The higher influence of the marine environment provokes bacterial inactivation of allochthonous micro biota from sewage and other discharges. Fecal contamination of marine environment from urban development and animals forming is increasing worldwide at an alarming rate. In many cases, rainfall can have a significant effect on indicator in recreational waters can be increased to high levels because animal / human wastes are washed from forest, land and urban setting. On a global basis, around 2 million deaths per year are attributed to water-borne diseases especially due to diarrhoea in children (Gordon *et al.*, 2004). Infections through recreational waters are not limited to enteric diseases but extend to the skin, ear, nose by throat and many microorganisms and they do not originate in the intestinal tract of warm blooded animals (Kumarasamy et al., 2009). *P. aeruginosa* in recreational water is harmful because it causes ear infections among bathers (Vignesh et al., 2013).

The emergence of antibiotic resistant bacteria is predictable in any environment where antibiotics are released. The occurrence of antibiotic resistant bacteria is also increasing in aquatic environments (Al-Bahry et al., 2009). The transfer of resistant bacteria to humans could occur via water and food if plants are watered with surface waters or sewage sludge, if manure is used as a fertilizer (Dolliver and Gupta, 2008). Bacterial resistance to antibiotics, due to the wide availability of antibiotics, and their improper usage and disposal, is an emerging public health concern. In the past, the uncontrolled and extensive use of pharmaceutical substances, mainly antibiotics, in human and veterinary medicine, animal husbandry, agriculture and aquaculture have increased the introduction of antimicrobial agents into the aquatic environment (Dang et al., 2008). Transmission of R-plasmid determinants may occur in less than one minute, and antibiotic resistance can spread rapidly among bacteria (Arvanitodou et al., 1997). Infections caused by resistant strains lead to a higher fatality rate than those caused by non-resistant strains, especially among immune-compromised individuals (Holmberg et al., 1984). The present study, therefore, investigates the prevalence of pollution indicators and multiple antibiotic resistances in the water and sediments of Nagappatinam coastal regions.

MATERIALS AND METHODS

Description of study site

The District of Nagapattinam has been carved out as a separate district due to bifurcation of Thanjavur district. According to this division, six taluks namely Sirkazhi, Tharangampadi, Mayiladuthurai, Valangaiman, Nagapattinam and Vedaranniyam were detached from their parent district (Thanjavur) to form this new district. The earlier history of this district is more or less the same as of its parent district, Thanjavur being its part till recently. The Nagapattinam district lies on the east coast to the south of Cuddalore district and another part of the Nagapattinam district lies to the south of Karaikkal and Tiruvarur districts. The district lays between 10.25º and 11.40º North Longitude and 76º 49º and 80.01º East longitude. In Nagapattinam coast zone there are two fishing hamlets, namely Akkaraipettai and Keechankuppam. There are no less than eleven ports on the coast Nagapattinam district, of which eight are open to foreign trades. The coastline has a number of harbors of which mention may be made of Nagore, Point Calimere, and Nagapattinam. The significant small ports are Kilvellore, Thirumulaivasalam, Nagapattinam, Velankanni, Topputturai, Muttupet and Adiramapatnam. The Nagapattinam district is made up the 6 Taluks of Nagapattinam, Kilvellore, Vedaranniyam, Mayiladuthurai, Sirkali and Thrangampadi. The East Side faces the Bay of Bengal. The district is the most part of a flat plain, slopping very gently to the sea on the east. The total geographical area of the district is about 3536.38 Sq.km. The coastal zone received enormous quantities of land materials, urban and rural sewage effluents, industrial and agricultural wastes throughout the year, especially during September to December, the months of intensive rainfalls.

In recent years, these sites undergo extensive changes due to industrialization, urbanization and the population pressure increased gradually. The prospected sites were located in areas with environments representing different vulnerability to contamination. Our study was focused on sea water and sediment used by an important population and which may be affected by anthropogenic disturbance and several sources. The south-west monsoon season prevails during June to August while the north-east monsoon season follows from September to December. The post monsoon season has a brief winter (January - February) and the summer (March – May) follows afterwards, characterized by warm humid conditions. Study area is Nagappatinam coastal region.

Sample collection

Water and sediment samples were collected from Nagappatinam beach region during July 2014 – August 2014. Approximately, 500 ml water samples and 200g sediment samples were collected with precautions and stored in an icebox, and transported to the laboratory to maintain the samples in refrigerator for microbiological analysis. The microbiological analysis was carried within 12 hrs.

Bacteriological analysis

All the specific media were prepared with the addition of old age sea water and autoclaved properly (Kumarasamy et al., 2009; Vignesh et al., 2013; Vignesh et al., 2014). The bacterial populations in different samples were estimated by spread plating method. In laboratory condition, 1.0 g of sediment samples were weighted, diluted in 9.0 ml of sterile distilled water and agitated twice in a cyclomixer for about 10 minutes and. Both water and sediment samples were serially diluted upto 10^{-5} dilution. Three replicates of 0.1/0.2 ml sediment/ water suspension from 10^{-3}, 10^{-4} and 10^{-5} dilution were inoculated on petridishes containing specific media as represented in Table 1.

Antibiotic susceptibility test

Sensibility tests to antimicrobials were performed using the agar disc diffusion methods from Kirby-Bauer, applied to muller hinton agar (MHA). The isolates were challenged with 10 antibiotics on Muller Hinton agar, and the results were interpreted based on the recommendations of the National Committee for Clinical Laboratory Standards for antimicrobial susceptibility tests (NCCLS, 1998). The 10 microgram of following antimicrobials: Ampicillin (AMP), Amoxycillin (AMX), Chloramphenicol (C), Ciprofloxacin (CIP), Erythromycin (E) 10, Gentamicin (GEN), Methicillin (MET), Penicillin - G (P), Tetracycline (TE) and Vancomycin (VA). The medium was dispensed on to separate presterilized petriplates under aseptic conditions. The solidified plates were swabbed with 24 h test organisms under aseptic condition. The antibiotic discs were placed individually using a sterile forceps on the inoculated agar plates and incubated for 24 h at 37 ± 1°C. After incubation, plates were observed for zones scale (Vignesh et al., 2012). All the trails were performed in triplicate and the mean values were noted.

RESULTS AND DISCUSSIONS

Pollution indicators

The aquatic environment consists of diverse microorganism and some of them have pathogenic characteristics. These bacteria were considered as etiological agents of infectious diseases to human and marine mammals (Baker-Austin et al., 2006). In this study, total viable counts (TVC) were in the order of enormity above 10^3 CFU/mL or CFU/g for both the samples (water and sediment) during July and August months. The bacteriological parameters were higher during August month than July month. However, the sea water samples contained higher pollution indicators during August while the July month water were also found to have high level of total coliforms, but less than August (TC) (<100 CFU/mL) (Figure 1). Sewage contamination in aquatic environments is commonly detected and quantified by enumerating the coliforms bacterial groups (Figure 2). Interestingly, apart from TVC and TC, the other bacterial parameters were also observed from the water and sediment samples of Nagappatinam coast. Commonly, higher count of both rod and cocci shaped colonies were observed from the total viable count plates on nutrient agar and their morphological characterization of bacterial isolates were recorded at table 2. The nil level was not found in July and August month in all the parameters. Some microbial pathogen in the coastal environment are indigenous to the ocean, including *Vibrio* whereas others like *E. coli, Salmonella sp., Shigella sp.,* are allochthonous which introduce through agricultural, urban surface run of waste water discharges and from domestic and wild animals (Vignesh et al., 2014). In July water samples, mean counts of TVC, TC, TS, TV, FC and VC were 101000 CFU/mL, 8600 CFU/mL, 980 CFU/mL, 1430 CFU/mL, 190 CFU/mL and 1170 CFU/mL, respectively (Figure 2). But in July sediment samples, mean counts of TVC, TC, TS, TV, FC and VC were 220000 CFU/mL, 19000 CFU/mL, 2700 CFU/mL, 2310 CFU/mL, 460 CFU/mL and 1900 CFU/mL, respectively.

Vignesh et al. (2014) observed that during rainfall, the microbial loads of running water may suddenly increase and reach reservoir bodies very quickly. However, most of the pollution indicators and human pathogenic bacteria counts are lower than those reported from the Bay of Bengal, Cauvery river and its estuary, Mondovi and Zuari estuary waters and Ganga waters

(Vignesh et al., 2013). The effect of rainfall on beach water quality can be quite dramatic. Owing to that, all the parameters were higher in August month compared to July months. Next to summer season, the premonsoon months such as July and August contains higher level of pollution indicators because more visitors and mass level of human and animals had been utilized these waters during this time. In August water samples, mean counts of TVC, TC, TS, TV, FC and VC were 136000 CFU/mL, 9500 CFU/mL, 940 CFU/mL, 1420 CFU/mL, 800 CFU/mL and 1100 CFU/mL, respectively (Figure 2). But in August sediment samples, mean counts of TVC, TC, TS, TV, FC and VC were 240000 CFU/mL, 21000 CFU/mL, 3000 CFU/mL, 3800 CFU/mL, 2000 CFU/mL and 3100 CFU/mL, respectively. The highest bacterial values can be attributed to the presence of large populations residing along the coastline. Developing countries where most of the residents lack access to suitable drinking water also obtain water for the daily needs of their residents from coastal ground water systems that are often contaminated (Vignesh et al., 2012). As human use of coastal land and water increases, so does the incidence of aquatic-borne disease from contact with contaminated water and eating contaminated shellfish (Kumarasamy et al., 2009).

Enteric microorganisms were mainly accumulated in the surface layers of sediments where the concentration of organism is 100 times greater than supernatant water (Pommepuy *et al.*, 1992). Sediments get resuspended due to various biogenic and physical disturbances. Higher counts of pathogenic pollution indicators were found in sediments rather than in water samples. Sediments may contain 100 to 1000 times the number of fecal indicator bacteria than the overlying water layers. In our study, the bacterial parameters were 2-5 fold higher in sediment than water samples. Unexpectedly, pathogenic microbes in most of the water/sediment samples of the present study were found to be above the prescribed limits (Vignesh et al., 2014), irrespective of the fact that we studied different locations of the Tamil Nadu coast where organic loads are introduced mainly by sewage outfall and human activities. Dominance of coliform bacteria in Tamil Nadu costal systems makes them useful microbial indicators. Higher bacterial population density in the sediments than water is generally due to the rich organic content of the former and the lesser residence time of the micro organisms in the water column than the sediments (Anon, 1997). The pathogens level was very high in August month compared to July month. The seasonal distribution pattern of different pathogenic bacteria were listed namely, TVC > TC > TV> TS > VC > FC/EC. The overall bacteriological characteristics observed for coastal samples were not good.

Antibiotic resistance study

Antimicrobial resistance patterns of the bacteria were not related to specific bacterial species or taxonomic groups. In general, evident differences in antimicrobial resistance patterns were observed among isolates from different sampling sites and different samples. According to previous report, high levels of antibiotic resistance in marine bacteria might be a result from non-indigenous bacteria with antibiotic resistant plasmids entering the seawater (Vignesh et al., 2012). The comparison of antibiotic resistant *Vibrio* sp. levels from the Nagappatinam coast (Table 3) (Figure 3). From the water sample, the resistance frequencies were very high: 76.7% of isolates in water sample and 93.3% in sediment sample. The 23.3 and 26.6 percentage of *Vibrio* were resistant to one antibiotic in water and sediment samples, respectively. In water, the highest double resistance and 3 antibiotic resistant were recorded in *Vibrio* sp such as 13.3% and 20.0%, respectively. 6.6% and 10.0% of *Vibrio* sp strains showed resistance to 4 and 6 antibiotics. None of the colonies from water sample of this site is resistant to 7-10 antibiotics while none of the colonies from sediment sample of this site is resistant to 8-10 antibiotics. Results also indicate that the water sample isolates showed a similar antimicrobial resistance profile to that of sediment samples. In sediment, 6.7% of *Vibrio* isolates were susceptible to all antibiotics. Of the total of 30 *Vibrio* isolates from water samples, 23.3% were susceptible to all antibiotics. Oliveira and Pinhata (2008) also observed the presence of resistant bacteria from human and animal origin in the Gonzaguinha and Ilha Porchat beaches, Brazil. Similar to that, Nagappatinam coastal ecosystem had high percentages of resistant organisms in sediment, showing thus a possible transfer of resistance factors among bacteria in these ecosystems.

The prevalence of these drug resistance bacteria requires reevaluation of water quality standards as well as more advanced purification of sewage prior to discharge into the environment (Grabow *et al*., 2000). Antibiotic resistance microorganism may be associated with reduced penetration of antibiotic into the cell or can result from active process such as changes in the transport of those compounds into or from bacterial cell. Moreover, plasmids that carry several different resistance genes can confer resistance to multiple antibiotics. Data on antibiotic resistant *E.coli* strains inhabiting Nagappatinam coast were given in Table 4 & Figure 4. The highest antibiotic resistance was observed in sediment samples than water samples. More than 60% was resistant to one or more of the tested antibiotics. In both water and sediment samples, low frequency of antibiotic resistance against 7-10 antibiotics was observed. In *E.coli* of sediment sample, 16.6% of isolates were resistant to 3 antibiotics and 10.0% were resistant to 4 antibiotics. In water sample, higher frequency of resistant was observed in 2 antibiotics (36.6%) while 80.0% of strains were susceptible to all antibiotics. The minimal percentage of these organisms showed resistance to 5–

10 antibiotics. The susceptible strains rates were high in both the samples. The highest frequency of susceptible was exhibited in *Vibrio* sp strains than *E.coli* strains.

Cross-resistance to several antibiotics may also occur when a resistance mechanism encoded by a single gene conveys resistance to more than one antibiotic compound (Baker-Austin *et al.,* 2006). Such transfer would contribute to the dissemination of resistance to antimicrobials agents (Oliveira and Pinhata, 2008). In fact, the transfer of resistance plasmid carrier in marine habitats was documented for several bacterial species (Sandaa, 1993). In regard to the antibiotic groups, the *Vibrio* sp and *E.coli* strains were resistant to 10 types of antibiotics (Table 5). The *Vibrio* sp., were resistant against 9 standard antibiotics such as Ampicillin (23.3 %), Amoxicillin (30 %), Chloramphenicol (0), Ciprofloxacin (1.6 %), Erythromycin (3.3 %), Gentamicin (5 %), Methicillin (10 %), Penicillin – G (40 %), Tetracycline (3.3 %) and Vancomycin (36.6 %) whereas at, *E coli* strains were resistant to 8 antibiotics - Ampicillin (25 %), Amoxicillin (13.3 %), Chloramphenicol (1.6), Ciprofloxacin (3.3 %), Erythromycin (6.6 %), Gentamicin (10 %), Methicillin (8.3 %), Penicillin – G (36.6 %), Tetracycline (6.6 %) and Vancomycin (33.3 %).

CONCLUSION

This study confirmed the presence of bacterial indicators of fecal origin at all the samples in each site of the study area. The Nagappatinam coastal areas were highly contaminated with pathogenic organisms such as *Vibrio* sp. and *E. coli*. In addition, the Fecal coliform (FC) ratio was found to be greatest due to human fecal contamination. Moreover, the water and sediment samples of Nagappatinam coastal areas showed the highest frequencies of resistance in relation to Penicillin-G and Vancomycin. Although, heterotrophic bacteria resistant to Amoxicillin, Ampicillin and Methicillin has also been observed. The strains were highly sensitive to Chloramphenicol, Ciprofloxacin and Tetracycline. The Nagappatinam coastal system facing severe anthropological activities, mostly due to industrial waste, sewage discharges dense population, mass bathing, tourisms and fishing activity etc. It is suggested that the inclusion of these groups could be made mandatory in coastal and estuarine waters for the environmental impact assessment. Based on the results it is concluded that the indication of the extent of microbial pollution in seawater and sediment areas may deteriorate the existing hygienic quality in the areas.

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REFERENCES

1. Al-Bahry, S., Mahmoud, I., Elshfie, A., Al-Harthy, A., Al-Ghafri, S., Al-Amri, I., Alkindi, A., 2009. Bacterial flora and antibiotic resistance from eggs of green turtles Chelonia mynas: An indication of polluted effluents. Marine Pollution Bulletin 58, 720–725.
2. Anon, J., 1997. Ecological, toxicological and environmental impacts assessment studies of the effluents discharge from MRLCHR in marine environmental of Nagapattinam, Tamil Nadu. *Ann. Rev. Microbiol.* 34: 357 - 263.
3. Arvanitodou, M., Tsakris, A., Constantindis, T.C., Katsouyannopulus, V.C., 1997. Transferable antibiotic resistance among Salmonella strains isolated from surface water. Water Research 37, 1112–1116.
4. Baker-Austin, C., M. Wright, R. Stephanauskas, and J.V. McArthur, 2006. Co-selection of antibiotic and metal resistance. *Trends. Microbiol.* 14: 176-182.
5. Clark, A., Torrey Turner., Padma Dorothy, K., Goutham, J., Kalavati, C., Bettaiya Rajanna., 2003. Health hazardsdue to pollution of watersalong the coast of Visakhapatnam, east coast of India. Ecotoxicology and Environmental Safety 56, 390–397.
6. Dang, H., Ren, J., Song, L., Sun, S., An, L., 2008. Diverse tetracycline resistant bacteria and resistance genes from coastal waters of Jiaozhou Bay. Microbial Ecology 55, 237–246.
7. Dolliver, H.A., Gupta, S.C., 2008. Antibiotic losses from unprotected manure stockpiles. J. Environ. Qual. 37, 1238–1244.
8. *Gordon, R., S. Kathleen-Black, and M. Finch, 2004. Detection and occurrence of waterborne bacterial and viral pathogens.* Wat. Environ. Res. *65: 23-32.*
9. Grabow, G.M., C. White, and J.L. Molwell, 2000. Heavy metal uptake by intact cells and protoplast of *Aurobasodium pullans. FEMS Microbiol. Ecol.* 45: 261-267.
10. Holmberg, S.D., Wells, J.G., Cohn, M.L., 1984. Animal to-man transmission of antimicrobial-resistant Salmonella: Investigation of U.S. outbreaks, 1971-1983. Science 225, 833-835.
11. Kocasoy, G., 1989. A method for prediction of extent of microbial pollution of seawater and carrying capacity of beaches. *Environ. Manage.* 13: 469–475.
12. Kumarasamy P, Vignesh S, Arthur James R, Muthukumar K, Rajendran A (2009) Enumeration and identification of pathogenic pollution indicators in Cauvery River, South India. *Research Journal of Microbiology* 4:540–549.
13. Mohamed, E., 2005. *Staphylococcus aureus* and fecal indicators in Egyptian coastal waters of Aqaba Gulf, Suez gulf and Red sea. *Egyptian J. Aqua. Res.* 31: 113-124.

14. NCCLS (National Committee for Clinical Laboratory Standards), 1998. Performance standards for antimicrobial susceptibility testing; Eighth Informational Supplement.NCCLS document M100-S8.Wayne, Pennsylvania.
15. Pommepuy, M., J.F. Guillaud, E. Dupray, A. Derrien, F. Le Guyader, and M. Cormier, 1992. Enteric bacteria survival factors. *Wat. Sci. Technol*. 25: 93–103.
16. Oliveira, A.J.F.C. and J.M.W. Pinhata, 2008. Antimicrobial resistance and species composition of *Enterococcus sp*., isolated from waters and sands of marine recreational beaches in Southeastern Brazil. *Wat. Resear*. 42: 2242–2250.
17. Small, C. and J.E. Cohen, 2004. Continental Physiography, Climate, and the Global Distribution of Human Population1. *Curr. Anthro*. 45: 2.
18. Sandaa, R.A., 1993. Transfer and maintenance of the plasmid RP4 in marine sediments. *Microbiol. Rele*. 2: 115–119.
19. Vignesh S, Muthukumar K, James RA (2012) Antibiotic resistant pathogens versus human impacts: A study from three eco-regions of the Chennai coast, southern India. *Marine Pollution Bulletin* 64:790–800.
20. Vignesh S, Muthukumar K, Santhosh Gokul M, Arthur James R (2013) Microbial pollution indicators in Cauvery river, southern India. In Mu. Ramkumar (Ed.), *On a Sustainable Future of the Earth's Natural Resources*, Springer earth system sciences, pp. 363–376. doi 10.1007/978-3-642-32917-3-20.
21. Vignesh S, Dahms HU, Emmanuel KV, Gokul MS, Muthukumar K, Kim BR, James RA (2014) Physicochemical parameters aid microbial community? A case study from marine recreational beaches, Southern India. *Environmental Monitoring and Assessment* 186(3):1875–1887.

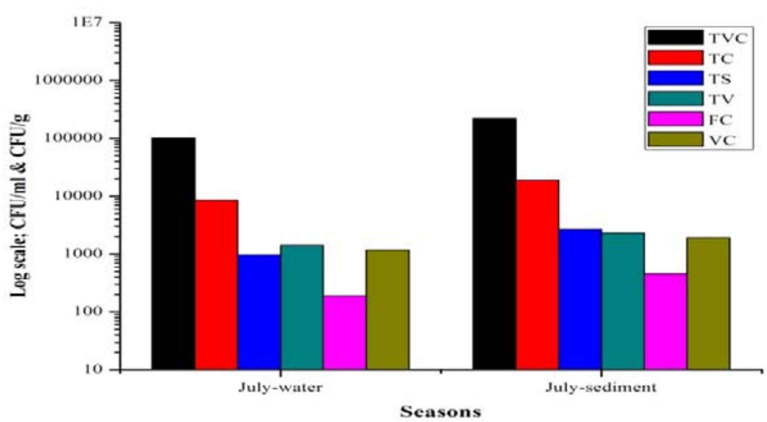

Figure 1. Pollution indicators in water and sediment samples in July month

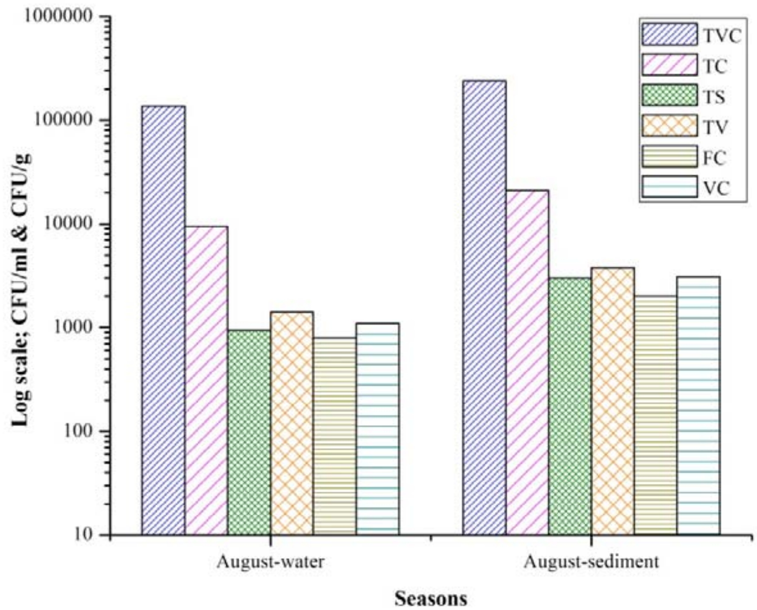

Figure 2. Pollution indicators in water and sediment samples in August month

Table 1. Details of specific culture media used for quantitative bacterial analysis

S.No	Bacterial indicators	Culture medium	Positive colonies	Incubation time	References
1	Total Viable Count (TVC)	Nutrient Agar	All colonies	37 °C ± 1°C for 24 to 48 h	Vignesh et al., 2012
2	Total Coliforms (TC)	MacConkey Agar	All colonies	37 °C ± 1°C for 24 to 48 h	Nagvenkar and Ramaiah, 2009
3	Total *Streptococci* (TS)	ME Agar	All colonies	37 °C ± 1°C for 24 to 48 h	Kumarasamy et al., 2009
4	Total *Vibrios* (TV)	TCBS Agar	All colonies	37 °C ± 1°C for 24 to 48 h	Vignesh et al., 2013
5	Fecal coliforms / *E.coli* (FC)	MFC Agar	Blue colonies	44.5 °C ± 1°C for 24 to 48 h	Clark et al., 2003
6	*Vibrio cholera* (VC)	TCBS Agar	Yellow colonies (>2 mm)	37 °C ± 1°C for 24 to 48 h	Vignesh et al., 2014

Table 2. Morphological characterization of bacterial isolates

S. No	Bacterial indicators	Observation		
		Gram Staining	Motility	Cell Shape
1	Total viable count	+ / -	+ / -	Rod / Cocci
2	Total coliform	-	+	Rod
3	Total *Streptococci*	+	-	Cocci
4	Total *Vibrio*	-	+	Curved Rod
5	Fecal coliform / *E.coli*	-	+	Rod
6	*Vibrio cholerae*	-	+	Curved Rod

Table 3. The antimicrobials resistant *Vibrio* strains and their susceptibilities, on sea water and sediment samples from

Nagappatinam coast areas

No. of Antimicrobials	*Vibrio* sp.					
	Water (n=30)		Sediment (n=30)		Total (n=60)	
	N	%	N	%	N	%
1 Antimicrobials	7	23.3	8	26.6	15	25
2 Antimicrobials	4	13.3	5	16.6	9	30
3 Antimicrobials	6	20	10	33.3	16	26.6
4 Antimicrobials	2	6.6	2	6.6	4	6.6
5 Antimicrobials	1	3.3	-	-	1	1.6
6 Antimicrobials	3	10	1	3.3	4	6.6
7 Antimicrobials	-	-	2	6.6	2	3.3
8 Antimicrobials	-	-	-	-	-	-
9 Antimicrobials	-	-	-	-	-	-
10 Antimicrobials	-	-	-	-	-	-
Total of Resistant	23	76.7	28	93.3	51	85
Total of Susceptibilities	7	23.3	2	6.7	9	15

N – Number of isolates

Table 4. The antimicrobials resistant *E. coli* strains and their susceptibilities, on sea water and sediment samples from Nagappatinam coast areas

No. of Antimicrobials	*E. coli*					
	Water (n=30)		Sediment (n=30)		Total (n=60)	
	N	%	N	N	%	N
1 Antimicrobials	7	23.3	8	26.6	15	25
2 Antimicrobials	11	36.6	8	26.6	19	31.6
3 Antimicrobials	3	10	5	16.6	8	13.3
4 Antimicrobials	2	6.6	3	10	5	8.3
5 Antimicrobials	-	-	1	3.3	1	1.6
6 Antimicrobials	1	3.3	1	3.3	2	3.3
7 Antimicrobials	-	-	-	-	-	-
8 Antimicrobials	-	-	-	-	-	-
9 Antimicrobials	-	-	-	-	-	-
10 Antimicrobials	-	-	-	-	-	-
Total of Resistant	24	80	26	86.7	50	83.3
Total of Susceptibilities	6	20	4	13.3	10	16.7

N – Number of isolates

Table 5. The resistance of *Vibrio* sp. and *E.coli* strains to specific antibiotics from Nagappatinam coastal area

S.No	Antimicrobials	Resistant Strains					
		Vibrio sp. (n=60)		*E.coli* (n=60)		Total (n=120)	
		N	%	N	%	N	%
1	Ampicillin (AMP)	14	23.3	15	25	29	24.1
2	Amoxicillin (AMX)	9	30	8	13.3	17	14.1
3	Chloramphenicol (C)	-	-	1	1.6	1	0.8
4	Ciprofloxacin (CIP)	1	1.6	2	3.3	3	2.5
5	Erythromycin (E)	2	3.3	4	6.6	6	5
6	Gentamicin (GEN)	3	5	6	10	9	7.5
7	Methicillin (MET)	6	10	5	8.3	11	9.16
8	Penicillin – G (P)	24	40	22	36.6	46	38.3
9	Tetracycline (TE)	2	3.3	4	6.6	6	5
10	Vancomycin (VA)	22	36.6	20	33.3	42	35

N – Number of isolates