Research Article

Studies on Cultural, Biochemical and Seasonal Variations of Bacterial Load from Coastal Waters of Bheemili, Visakhapatnam

Prasanna Lakshmi, G., Naresh, M., Pavan Kumar, K. and Ramesh Babu, K.

Department of Marine Living Resources, College of Science and Technology, Andhra University

Corresponding Author Email: gnana.gorle@gmail.com

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Abstract: The microbial status in the coastal waters was studied at the Bheemili of Visakhapatnam. The study mainly focused on the bacterial load, abundance and their culture characteristics. For enumeration of bacteria spread plate method was employed. IMViC biochemical tests were conducted to identify the bacteria. Different pathogenic bacteria were identified as *Vibrio, Enterococcus, Salmonella, Staphylococcus, Pseudomonas, Shigella, E. coli and Bacillus species*. The highest distribution of pathogenic bacteria was observed during the summer months due to the discriminate discharge of sewage and urban runoffs. The result of the present study provides the insight into occurrence and distribution of pathogenic bacteria in coastal waters may be attributed due to the rapid industrialization, urbanization, raw sewage and also influence of anthropogenic inputs into inshore and offshore waters. Hence the continuous monitoring of coastal water is very much important with reference to bacterial population is concerned.

Keywords: IMViC biochemical tests, urban runoffs, Pathogenic bacteria.

Introduction

Oceans are the largest water bodies which have been polluted over the past decades with excess human activities have extremely affected the marine environment by continuous discharge of untreated sewage waste, untreated industrial wastes; agricultural wastes to distressing level causes threat not exclusivelyto marine environment but also public health and declining the aesthetic values of the coastal areas. Marine pollution became a worldwide environmental concern (Kennish, 1997). Innovate research have been implemented relating to marine life and human health to understand the disease causing organisms in coastal ecosystems. Coastal areas are important regions of the marine environment which in particular shelf areas, embayment and estuaries are often benefitted from flow of nutrients from land to the coastal waters. The wastes and land run-offs carry high levels of pathogenic bacteria into the marine environment, which causes a negative impact on the marine environment and public health. The microorganisms exhibit heterotrophic link between particles and dissolved oxygen matter in oceanic and coastal waters by influencing nutrient cycles. The physic-chemical parameters play a major role in influencing the bacterial communities and their viability (Jalal *et al.*, 2010).

The characteristics of microbial ecology were determined by defining the population dynamics, physic-chemical characteristics and metabolic processes of the microenvironment (Chui wei Bong and ChoonWeng Lee, 2008). The evolution of natural and anthropogenic fluxes determined the ecosystem of the coastal waters of Visakhapatnam. The present study is to investigate the abundance and enumeration of pathogenic bacteria.

Materials and Methods

Study area: Visakhapatnam coast is located at latitude 17° 40' 48.32" N and longitude 83° 12' 5.80" E of the Bay of Bengal. Bheemili shore waters were sampled.

Sample collection

The sampling was done seasonal wise from March 2017 to February 2018.For bacteriological analysis. Surface sea water was collected into glass bottles and immediately transferred to the laboratory.

Culture media

Zobell marine agar was used for the isolation of bacterial cultures from samples. Other selective media like MacConkey agar, Mannitol Salt Agar, TCBS agar, etc., were used for the identification of bacterial cultures.

Bacteriological analysis

1 mL of water samples was serially diluted and dilutions of 10⁻², 10⁻³ and 10⁻⁵ were plated on Zobell marine agar by spread plate method and incubated for 34°C for 24h. The culture characteristics were studies for isolated colonies and were re-streaked on to the Zobell marine agar plates and were incubated at 32°C for 24h. The cultures were subjected to Gram's staining and other biochemical tests for further identification.

Results and Discussions

Total of 12 different bacterial strains were isolated during the study period from the coastal waters of Bheemili. Bacteria were isolated by subjecting water sample to serial dilution and the bacteria were identified as per Bergey's manual of systematic bacteriology. In the water sample gram-negative bacteria are abundant as compared to gram-positive bacteria. Coastal waters have been polluted by microbial pathogens which are introduced by urban run offs, industrial and agricultural discharges, domestic wastes. The coastal waters have been polluted by continuous discharge of untreated sewage waters and industrial effluents causing hazardous effect to marine habitat and significance of coastal areas (Aneela shaheen, 2016).

Total 12 different bacterial strains were isolated and identified by applying various biochemical tests (table 1). The gram-negative bacteria isolated from water sample includes *E. coli, Pseudomonas aeruginosa, Vibrio parahaemolyticus, Vibrio cholera, Salmonella typhimurium, Proteus vulgaris, Enterobacter aerogenes* and *Shigella*. The gram-positive bacteria are *Bacillus subtilis, Enterococcus faecalis, Bacillus cereus and Staphylococcus aureus*.

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Biochemi	Ec	Vc	Vp	Pa	Sh	St	Pv	Ea	Bs	Bc	Ef	Sa
-cal												
tests												
Catalase	+	+	+	+	-	+	+	+	+	+	-	+
Oxidase	-	+	+	+	-	-	-	+		-	-	-
Motility	+	+	+	+	-	+	+	+	+	+	-	-
Indole	+	+	+	-	-	-	+	-	-	-	-	-
H2S	-	-	-	-	-	+	+	-		-	-	-
Gas	+	-	-	+	-	-		+	-	+		-
Citrate	-	+	-	+	-	+	+	+	+	+	-	+
MR	+	-	+	-	-	+	+	-	-	-	-	+
VP	-		-	-	-	-	+	+	+	+	+	+
Glucose	+	+	+	_	+	+	+	+	+	+	+	+

Table 1. Biochemical test results for isolated bacteria from March 2017- February 2018

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					1	1	1	1	1			
Lactose	+		-	-	-	-	-	+		-	+	+
Starch	-			-	+	-	-	-	+	+	-	-
TSI	A/A	K/A	K/A	K/K	A/-	K/A	A/A	K/A	-	K/K	A/A	-
Mac	Pink	Pale	Pale	Brow	Flat	Pale	Yello	Pale	-	-	Red	-
Conkey				n	colo		w					
					ny		media					
MSA	-	Yello	Yell	-	-	-	-	-	-	Pink	Yell-	Yell-
		-W	-OW								ow	ow

Note: Ec=*E.coli*, Vc=*Vibrio cholera*, Vp=*Vibrio parahaemolyticus*, Pa=*Pseudomonas aeruginosa*, Sh=*Shigella*, St=*Salmonella typhimurium*, Pv=*Proteus vulgaris*, Ea=*Enterobacteraerogenes*, Bs=*Bacillus subtilis*, Bc=*Bacillus cereus*, Ef=*Enterococcus faecalis*, Sa=*Staphylococcus aureus*; A=acid, K=alkaline



Figure 1. Abundance of isolated bacteria in summer







Figure 3. Abundance of isolated bacteria in post monsoon



Figure 4. Abundance of isolated bacteria in winter

By the enumeration of microbial status on Bheemili coastal waters for summer, monsoon, post monsoon, winter seasons. Total of 10 bacterial strains were isolated during summer (Figure 1), with high abundance of *Enterococcus faecalis* (29.6%) and low abundance of *Vibrio cholera* (2.36%).

8 bacterial strains were isolated during monsoon (Figure 2), with high abundance of *Bacillus cereus* (34.15) and lowest abundance of *E. coli* (2.24%).

11 bacterial strains were isolated during post monsoon (Figure 3), with high abundance of *Salmonella typhimurium* (19.74) and low abundance of *Enterobacter aerogenes* (1.48).

10 bacterial strains were isolated during winter (Figure 4), with high abundance of *Enterococcus faecalis* (27.35%) and low abundance of *Bacillus subtilis* (1.74%).

The accumulation of these bacteria indicates high loads of organic effluents with domestic sewage and urban runoffs. The accumulation of contaminants can cause biomagnifications by increasing BOD, COD, heavy metals and faecal coliforms that affect human being.

Enterococcus faecalis is gram-positive cocci, non-motile, non-capsulated bacteria, which is a commensal bacterium, inhabits gastrointestinal tracts of human. It is highly observed in winter and summer seasons. It causes endocarditis, urinary tract infections, meningitis and other infections in humans. This bacterium can tolerate 60°C for 30 minutes and showed high antibiotic resistance upon various antibiotics (Esrafil Balaei Gajan*et al.*, 2013).

Bacillus cereus is gram-positive bacteria, facultative anaerobes and produce endospores. The abundance of these bacteria accumulates into molluscan shells and cause food-borne illness with symptoms of severe nausea, vomiting and diarrhea. These bacteria showed high abundance during monsoon.

The accumulation of these bacteria due to the contamination of shore waters with non-treated sewage water. *Salmonella typhimurium* is a gram-negative bacterium. It is mostly found in intestinal lumen. It causes gastroenteritis, diarrhea and typhoid fever in humans. It is mostly observed in post-monsoon months.

The accumulation of these pathogenic bacteria in shore waters is caused by dumping the untreated sewage waters, house effluents and municipal wastes. The preponderance of *E. coli* and *Vibrio cholerae* were highly accumulated due to the discharge of sewage pollution. The survival and abidance of these bacteria may cause risk to public health (Abhirosh Chandran, 2008). The pathogenic bacteria isolated from the brackish waters of Chilika lagoon were subjected to morphological, biochemical, physiological and serological characters revealed that they hold strong potential for development of anti-pathogenic agents (Subhashree Parida, 2012).

Conclusion

The present study showed that the coastal waters are polluted with local and urban runoffs and they are not safe for public. The sewage was directly discharged into the rivers and coastal habitats, which leading to the accumulation of pathogenic bacteria and causing contagious diseases. The contamination of shore waters controlled by improved means of sewage collection and treatment.

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Conflicts of interest: The authors declare that they have no competing interest.

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