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Current Scenario on Enteric Pathogens from the Coastal Area off Visakhapatnam, India

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Abstract: The current study aimed to establish information on enteric pathogens from coastal waters of Visakhapatnam. Eight different species were identified and they are *Escherichia coli*, *Enterococcus species, Shigella species, Salmonella species*, *Proteus/Klebsiella species*, *Vibrio species*, *Faecal Streptococci*, and *Pseudomonas species*. The colony count values varied from three different stations for each bacterial species and are represented in CFU/ml. The colony count values for *Faecal coliform* ranged from 7 to 39 CFU/ml, 5 to 28CFU/ml for *Enterococcus sps*, 10 to 23 CFU/ml for *Shigella sps*, 5 to 30 CFU/ml for *Salmonella sps*, 12 to 50 CFU/ml *Proteus/ Klebsiella sps*, 13 to 55 CFU/ml for *Vibrio sps*, 5 to 30 CFU/ml *Faecal streptococcus*, 3 to 28 CFU/ml for *Pseudomonas sps* respectively. Total viable counts varied from 120 to 812 CFU/ml and Total count varied from 15 to 92 CFU/ml. There was a significant difference found in enteric pathogens from three different stations.

Key words: Colony count, Enteric pathogens, *Enterococcus, Pseudomonas, Streptococcus*.

Introduction

Microorganisms which are inhabited in sea water can cause dangerous diseases like diarrhoea and cholera and have serious and harmful impacts on human health (Buras *et al.,* 1987; Reeves *et al.,* 2004). The contribution of harmful pathogens at coastal areas was aided by domestic waste and other organic waste materials. Seawaters, sediments and estuaries, and hydrothermal vents are rich with microbial loads and microorganisms play key role in decomposition of organic matter into inorganic matter and helps in cycling of nutrients which will beneficial to the all living components in oceans (Cevera *et al.,* 2005). In general, human health problems are mainly governed by intake of sea foods and sea bating at coastal contaminated sites by urban discharges in to the sea (Shuval 1999). Sea water enriched with contaminants due to direct discharge of waste water from communities and streams, runoff of rivers (Shuval 2005). Vegetable wastes and faecal matters are chief source of microorganisms which transferred to the sea during course of time (Sharma and Chaturvedi 2007; Williams *et al.,* 2007). Generally majority of the *Vibrios* are indigenous to oceans. Agriculture, surface runoff and waste water discharge are the agents which leads to introduction of allochthonous species like *Escherichia coli, Salmonella spp*, and *Shigella spp* in to the marine environment. Majority of the *Vibrios* and *Salmonella spp*. are dangerous to humans and some leads to fatal infestations (Blake *et al.,* 1980; Grimes 1975; Carlson *et al.,* 1968; Gerba and Schaiberger 1975). The present study focused on the determination of enteric pathogens at coastal waters of Visakhapatnam.

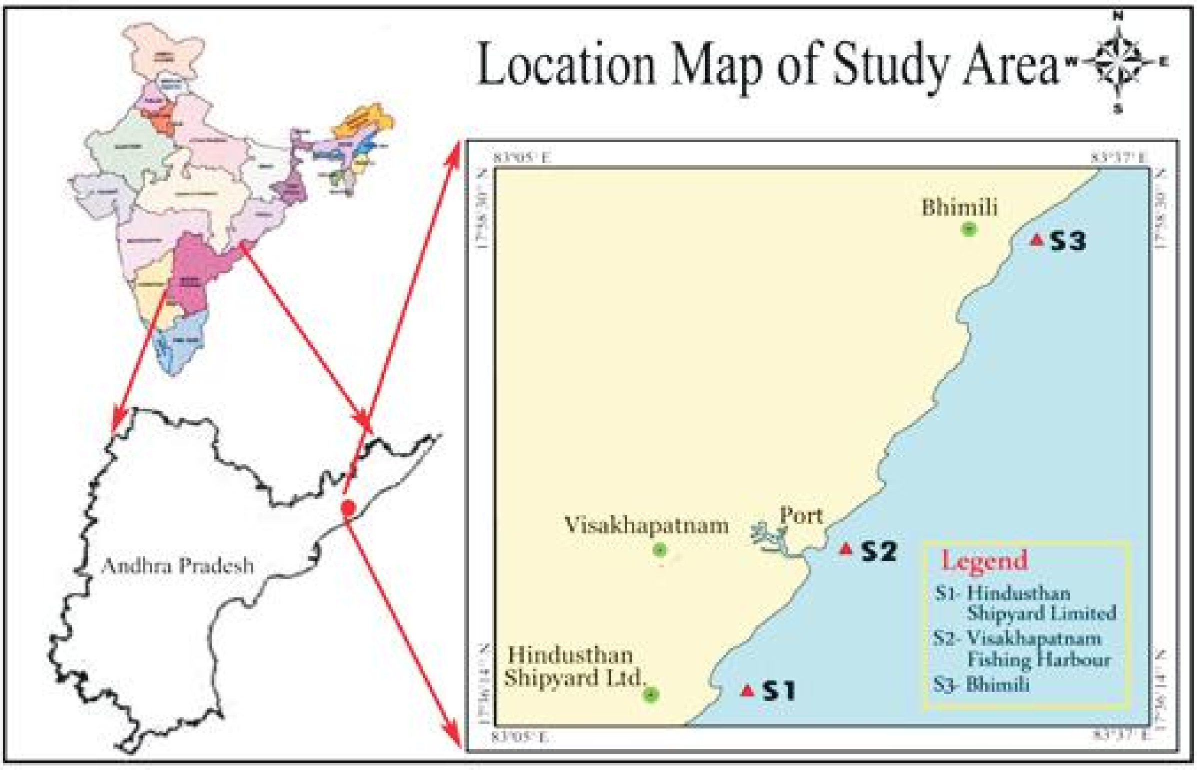
Materials and Methods

Study area:

The current study includes three stations. Station-I lies on the east coast between the latitude 17^0 53' 25" N and 83^0 28' 30"E longitudes. Station-II lies on the east coast between the latitude17^0 41' 17" N and 83^0 19' 10" E longitudes. Station –III lies on the east coast between the latitude 17^0 36' 58" N and 83^0 15' 26"E longitude shown in **Fig. 1**. The water samples were collected from each station for a period of one year from March 2012 to February 2013. The physic-chemical parameters of the collected samples were recorded. The temperature was recorded using a thermometer (1-51^{0}C range with ± 0.1^0 Brannan, UK). For dissolved oxygen water samples were collected in 2 lit PVC bottles and maintained ice conditions, brought to the laboratory for analysis. Dissolved oxygen (DO) was estimated by Winkler's method (Grasshoff *et al.,* 1999). The salinity of the samples were estimated by using digital Auto Salinometer (Model, TSK, accuracy ± 0.001) and the pH using an ELICO LI 610 pH meter (accuracy ± 0.01).

The selective media were used for the growth of eight different bacterial species identified at east coastal waters of Visakhapatnam. Membranes filter (MFC) Coliform for *faecal coliforms*, M-7h-FC agar for *Escherichia coli,* Xylose lysine Deoxycholate Agar for *Shigella sps*. and *Salmonella sps,* Thiosulphate Citrate Bile Sucrose (TCBS Agar) for *Vibrio sps,* Enterococcus Agar for *Enterococcus sps*, Cetrimide Agar for *Proteus sps*, Nutrient agar for TVC count. The spread plate technique was employed for enumerate the enteric bacterial species. The results were tabulated as Colony forming units (CFU/ml). All the chemicals and media used for (bacterial enumeration) the current experiment purchased from Hi-media, Visakhapatnam.

Fig. 1: Study area map showing the three sampling stations at coastal waters of Visakhapatnam



Results

Physicochemical Parameters

Temperature

The water temperature from three sampling stations varied from 26°C to 34°C. The higher temperature 34°C was recorded at Bhimili (station III) in April month and the lower temperature 26°C recorded at Hindustan shipyard Station I. The variation in water temperature was mainly due to prevailing weather condition.

pH

The pH value from three sampling stations varied from 7.7 to 8.3. The higher pH 8.3 was recorded at station III in July (Bhimili), the lower pH 7.7 was recorded in station II in July (Visakhapatnam fishing harbour). There were no significant differences in pH values for all sampling stations. The standard pH for sea water ranged from 6.5 to 8.5 (DOE 2006), the pH values obtained from these stations were within the recommended standard.

Salinity

The salinity value recorded from the three sampling stations varied from 23 to 33ppm. The maximum salinity value was recorded as 33ppm at station III in April month (Bhimili) and the minimum salinity value was recorded as 23ppm at station II in December (Visakhapatnam fishing harbour). Lower salinity values might be due to the continuous inflow of fresh water.

Dissolved Oxygen (DO)

The Dissolved Oxygen (DO) values noticed from three stations varied from 3.3 to 6.2 mg/ml. The higher D.O value was recorded as 6.2mg/ml at station II in August (Visakhapatnam fishing harbour) and the lower D.O value was recorded as 3.3mg/ml at station II in September (Visakhapatnam fishing harbour). Generally the dissolved oxygen will be affected by the water temperature, tides and water depth.

Enteric Pathogens

Total Viable Count (TVC)

The total viable count (TVC) varied from 120 to 812 CFU/ml in three different sampling stations. The maximum and minimum Total viable Bacterial count was recorded at station II (Table 2)

Total Count (TC)

The total count (TC) varied from 15 to 92 CFU/ml in three different sampling stations. The maximum total count (92 CPU/ml) was registered at station II in April (Table 2) and minimum total count (15 CPU/ml) was recorded at station III in May (Table 3).

Faecal coliform **(FC)**

The number of *faecal coliform* varied from 7 to 39 CFU/ml in three sampling stations. The higher number of *faecal coliform* (39 CFU/ml) was observed at station II in November (Table 2) and lower number of *faecal coliform* (7CFU/ml) was observed at station III in June (Table 3).

Enterococcus colonies (EC)

The number of *Enterococcus species* varied from 5 to 28 CFU/ml in three sampling stations. The higher number of *Enterococcus species* (28 CFU/ml) were observed at station II in January (Table 2) and lower number of *Enterococcus species* (5 CFU/ml) were observed at station III in April (Table 3)

Shigella species **(SH)**

The *Shigella species* colony count varied from 10 to 23 CFU/ml in three sampling stations. The maximum number of *Shigella species* (23 CFU/ml) were observed at station II January (Table 2) and minimum *Shigella species* (7 CFU/ml) were recorded in station III in May (Table 3).

Salmonella species **(SA)**

The *Salmonella species* colony count varied from 5 to 30 CFU/ml in three sampling stations. The Higher number of *Salmonella species* were observed (30 CFU/ml) at station II January (Table 2) and lower *Salmonella species* were recorded 5 CFU/ml in station III in May (Table 3)

Proteus/Klebsiella species **(P/K)**

The *Proteus/Klebsiella species* varied from 12 to 50 CFU/ml in three sampling stations. The maximum *Proteus/Klebsiella species* were observed (30 CFU/ml) at station II November (Table 2) and minimum *Proteus/Klebsiella species* were observed (12 CFU/ml) in station III in May (Table 3).

Total *Vibrio species count* (TV)

The total *Vibrio species* count varied from13 to 55 CFU/ml in three different sampling stations. The Higher number of *Vibrio species* were observed (35 CFU/ml) at station II April (Table 2) and lower *Vibrio species* were observed (13 CFU/ml) at station III in April (Table 3).

Faecal streptococcus **(FS)**

The faecal *Streptococcus species* count varied from 5 to 30 CFU/ml in three different sampling stations. The Higher number of *faecal streptococcus species* were observed (30 CFU/ml) at station II in December (Table 2) and lower *faecal streptococcus species* were observed (5 CFU/ml) in station III in April (Table 3).

Pseudomonas species **(PA)**

The *Pseudomonas species* count varies from 3 to 28 CFU/ml in three different sampling stations. The maximum *Pseudomonas species* count were observed (28 CFU/ml) at station II in December (Table 2) and minimum *Pseudomonas species* were observed (5 CFU/ml) at station III in April (Table 3).

Table 1: Monthly colony count (CFU/ml) variation in station-I from March- 2012 to February 2013

MONTH	TVC	TC	FC	EC	SH	SA	P/K	TV	FS	PA
MAR	149	38	18	10	11	13	20	35	12	14
APR	138	40	19	9	13	14	25	45	10	15
MAY	149	30	15	10	10	15	20	43	15	17
JUN	250	32	14	10	12	13	19	38	13	13
JUL	360	58	19	14	11	12	22	38	16	10
AUG	480	65	13	9	11	15	17	23	10	15
SEP	685	85	28	15	11	13	20	35	10	15
OCT	490	93	18	17	15	18	27	23	15	17
NOV	390	85	39	15	12	18	27	49	16	17
DEC	625	83	14	15	15	15	15	22	17	17
JAN	685	85	19	15	12	18	17	49	15	18
FEB	650	85	20	15	12	15	19	45	16	18
MEAN	420.9	64.9	19.6	12.8	12.0	14.9	20.6	37.8	13.8	14.5
S.D	213.9	24.16	7.25	2.94	1.56	2.10	3.89	9.90	2.58	4.79

Table 2: Monthly colony count (CFU/ml) variation in station-II from March- 2012 to February 2013

MONTH	TVC	TC	FC	EC	SH	SA	P/K	TV	FS	PA
MAR	185	43	15	8	10	10	20	42	12	13
APR	190	48	15	8	10	18	20	47	9	11
MAY	218	40	16	11	12	15	21	43	8	15
JUN	229	38	14	10	17	13	25	39	15	13
JUL	335	35	13	15	20	13	37	45	17	18
AUG	446	29	17	14	17	30	29	38	23	17
SEP	730	36	12	14	15	27	40	40	25	14
OCT	812	52	17	17	13	23	43	55	27	13
NOV	739	39	18	19	13	22	50	49	28	17
DEC	749	42	18	28	20	23	27	36	30	17
JAN	450	39	28	28	23	30	30	49	30	20
FEB	550	40	20	25	20	28	29	40	26	17
MEAN	469.41	40.08	16.91	16	15.83	21	30.91	43.5	20.6	25.41
S.D	241.50	5.94	4.16	6.82	4.32	7.07	9.66	5.56	8.4	33.97

Table 3: Monthly colony count (CFU/ml) variation in station-III from March- 2012 to February 2013

MONTH	TVC	TC	FC	EC	SH	SA	P/K	TV	FS	PA
MAR	130	20	10	5	6	7	14	20	4	4
APR	120	19	10	5	5	8	15	19	5	3
MAY	139	15	8	7	3	5	12	17	8	5
JUN	145	13	7	7	8	10	11	13	6	8
JUL	173	25	8	10	10	8	14	15	8	5
AUG	253	28	10	8	9	10	10	15	6	10
SEP	253	48	12	10	11	10	13	15	9	8
OCT	169	28	15	7	9	13	20	20	9	10
NOV	170	39	11	11	8	11	20	21	10	3
DEC	189	35	20	9	10	10	15	19	9	9
JAN	140	39	22	7	9	13	15	19	13	10
FEB	180	40	23	8	10	13	17	20	12	13
MEAN	167.5	29.08	13	7.83	8.16	9.83	15.5	17.75	8.25	7.33
S.D	44.98	11.15	5.56	1.89	2.36	2.51	4.73	2.63	2.70	3.25

Discussion

The present investigation represents that the presence of 8 different enteric pathogenic microorganisms along the coastal waters of Visakhapatnam. The bacterial strains include the number of *Total coliform* (TC), *Faecal coliform* (FC), *Faecal Streptococcus*(FS), *Salmonella species* (SA), *Shigella species* (SA), *Total Vibrio species* (TV), *Proteus/Klebisella species* (K/P) and *Pseudomonas species* (PA).The guidelines for recreational marine water recommended or implemented by various US governmental agencies were at geometric mean 1000/100ml of total *coliform* (U.S.EPA 1986). There was a strong and positive correlation between *total coliform* & *faecal coliform* in all sampling stations.

According to marine water quality standards (IMWQS) (DOE 2006), the acceptable *E.coli* count was 100MPN/100ml or 200CFU/100ml by U.S.EPA guidelines (U.S.EPA 1986). Based on these standards the sea water at three selected locations for current study was not safe for body contact and shellfish activities. Countries such as

United States, Philippines & Canada introduced standards for *E. coli* for recreational seawater at maximum geometric mean of 200 counts/100ml water, while in Australia and Hong Kong at 150&180counts/100ml, respectively (Wang, 1999).

In addition to *Faecal coliform* and *total coliform*, *Faecal Streptococci* was also used as a indicator of microbial pollution in sea water because *Enterococci* are more resistant to physicochemical and environmental stress than *E.coli* (U.S.EPA 1986; EU 2006).The standards recommended by US Environmental Protection agency (U.S.EPA 1986) for marine water as recreational purposes / bathing was 35cfu/100ml. While according to WHO standards (2003) for category B water the intestinal *Enterococci* was <40 per 100ml of water. Based on these standards all the three different stations were showed higher number of colony count. The maximum number of *Faecal Streptococci* was related to soil erosion, which can cause a high turbidity of water, sunlight will reduce the activity of *Faecal Streptococci* survival (Robert et al., 1994) and in this study the number of *Faecal Streptococci* inversely proportionally with turbidity. According to Fujioka *et al.,* (1981), the presence of sunlight is a major factor controlling the survival of *Faecal coliform* and *Faecal Streptococci* in sea water. Elmanama *et al.,* (2005) found a significant correlation between *Faecal coliform* and *streptococci* and between *salmonella* and *Vibrio* at Gaze beach contaminated with sewage outfall.

Salmonella and *Shigella species* were pathogenic that are distributed worldwide and transmitted mainly through food and water ingestion, their presence in water including recreational water, render that water unfit for human use (Elmanama, 2005). The maximum counts of *shigella species* were found in fishing harbour at Visakhapatnam and Hindustan shipyard. The higher *salmonella species* were found in fishing harbour close to the shore.

Vibrio includes several species, higher number of *Vibrio species* are found in Visakhapatnam fishing harbour. Water close to the shore contains high number of *Vibrio* count than to the offshore. Coastal water close to shore contains more counts than the offshore. Pradeep *et al.,* (1984) 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.,* (1986) has reported that zooplankton can enrich *Vibrio parahaemolyticus* from their ambient environment.

According to Banat et al., (1998) the number of total *coliform* was higher, when the temperature was at 30°C at around 650/100ml. On the other hand, *coliform* were more rapidly inactivated in sunlight than in the dark. The solar radiation was found to be the most significant factor affecting the mortality of *coliform* bacteria (Yukselen *et al.,* 2003).

Higher numbers of *Proteus /Klebsiella* species counts were found in fishing harbour. The recreational water is harmful because it caused ear infections among the bather. The variation of results existed may depend upon location of the study, sourced of pollutants, climate changes, and topography of the beach etc.

Present study showed that all the bacteriological parameters *total coliform*, *Faecal coliform* and *Faecal Streptococci* counts showed above the recommended standards for sea water for recreational purposes. The presence of these faecal indicators was due to the existence of many sewage outfalls along the beach, which carried effluent from domestic dwelling and hostel. At station II the garbage was located of these factors contributed to the presence of high faecal bacteria in the water. The Visakhapatnam fishing harbour is busiest waterways compared to the Hindustan shipyard and Bhimili. , fishing harbour activities and many ships and tankers do their cleaning at the sea this oil contaminated wastewater is brought up to the beach during the low tide.

Conclusion

The current findings suggest that among three different stations Visakhapatnam fishing harbour contains greater amount of enteric pathogens compared to Hindustan shipyard and Bhimili. The coastal waters of Visakhapatnam being a dumping site of the sewage discharge, untreated industrial effluents, fishing activities, promote the harmful pathogenic count particularly at onshore waters. The coastal area contamination due to enteric pathogens leads to quality and quantity deterioration of the marine valuable resources. A local community depends on the marine resources for their livelihood, will suffer due to microbial contamination of the resources and finally leads to severe economic losses. It can be concluded that Visakhapatnam harbour (Station 2), is the contaminated site enriched by enteric pathogens.

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