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RESEARCH ARTICLE

BACTERIA OF THE RECREATIONAL BEACH WATERS OF VISAKHAPATNAM, INDIA

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ABSTRACT

Bacteria of the beach waters at five sampling stations [Rama Krishna beach (RKB-I; RKB-II), Rushikonda beach, Tennati park and Aqua Sports complex] of Visakhapatnam, Andhra Pradesh were studied. Total coliforms, faecal coliforms and *Enterococci* were confirmed as the indicators. The incidence of *Vibrio cholera* in beach waters was also studied. The MPN value of coliforms at Rama Krishna beach-I & Tenneti Park was 160 whereas at Rama Krishna beach-II & Rushikonda it was 180+. Members of *Enterobacteriaceae* were higher than the fecal *Streptococci*. The water of the aqua sports complex was completely free from bacteria. No *V. cholera* was found at all the stations.

INTRODUCTION

Microbes are closely associated with the human habitation and some are beneficial while others are harmful (Pelczar, 2002). Microorganisms and their activities in the fresh, estuarine and marine waters have been reported by Pelczar (2002). The coastal areas are known for the enormous source of food and recreation (Pelczar, 2002). Enormous loads of organic, inorganic, sewages and untreated effluents are in fluxed into the coastal waters due to recreational activities, industrialization and urbanization. These inputs results in outburst of microbial loads besides spoiling the water quality. Among the microbes some are hazardous and others are non-hazardous and serve as indicator species as per example *Escherichia coli* and *Streptococci* in aquatic environment indicate the extent of sewage pollution and portrays the sensibility of the environment. Bacteria like total coliforms, coliforms, *E. coli*, *Streptococci* etc. serve as indicator organisms to reveal the extent of pollution.

Some of the researchers who carried out the microbial loads in the beach waters include Clark Turner *et al*, (1985), Fuhrman *et al*, (1999), Ifremer and Jiang (2000), Jiang (2001), Namaihira *et al*, (2003), Mc Bride *et al*, (2005), Sugumar *et al* (2008), Ajit Kumar *et al* (2009), Ayokunle *et al* (2012), Brilliant *et al* (2013), Rajagopal *et al* (2013), Ramesh Babu *et al* (2014), Ranjith and Nagamurugan (2014), Maloo *et al* (2014), Viswanadham and Kondalarao (2015), Sunil Borade *et al* (2015), Viswanadham and Kondalaarao (2015) etc. The present work aims at the biological indicators like total coliforms, fecal coliforms, enterococci, *E. coli* and *Streptococci*, *Enterobacteriaceae* and *Vibrio cholerae* of the recreational beach waters of Visakhapatnam, Andhra Pradesh (India) at five sampling stations: RKB – I, RKB -II, Rushikonda Beach, Tenneti Park and Aqua Sports complex.

MATERIALS AND METHODS

Water samples were collected at five sampling stations– RKB-I (17°42'43.16"N, 83°19'12.55"E), RKB–II (17°42'37.03"N, 83°19'4.15"E), Aqua sports complex (ASC, 17°42'59.65"N, 83°19'30.80"E), Tenneti Park (TP, 17°44'51.69"N, 83°21'3.26"E) and Rushikonda Beach (RuKB, 17°46'52.98"N, 83°23'8.84"E) of Visakhapatnam coast (Fig 1). Sea water samples were collected from the surf beaten zone in sterilized glass bottles. Bacteria of the water samples were cultured and the bacteria were analyzed for total coliform bacteria, coliform bacteria, *E. coli* and *Streptococci*, *Enterobacteriaceae and Vibrio cholerae* by following methods of Surendran *et al.*, (2006).

Total coliform bacteria, coliform bacteria and *E. coli* were enumerated by three tube Most Probable Number (MPN)

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technique (Speck, 1976). After 24 hr of incubation at 37^0C in lauryl sulphate tryptose broth (LSTB), tubes with turbidity and gas production were recorded as total coliform bacteria and a loopful of culture from the positive tubes were transferred into EC broth. Tubes with turbidity and gas production were recorded as coliform bacteria after 24 hr of incubation at 44.5 ± 0.5^0C. From the positive tubes of EC broth a loopful of culture was streaked on eosine methylene blue (EMB) agar and colonies showing metallic sheen were recorded as presumptive *E. coli*. Further, typical colonies on EMB agar were purified and subjected to indole, methyl red, Voges- Proskauer and citrate utilization tests (IMViC) of Pelczar (2002) and confirmed *E. coli*. Pour plate technique using Kenner Fecal (KF) agar was employed for the estimation of streptococcal count and typical colonies were counted after incubation at 37^0C for 48 hr and expressed as colony forming unit (CFU) per ml or g of the sample.

For the detection of *Enterobacteriaceae,* Violet Red Bile Glucose Agar (VRBGA) was taken in a flask, melted on a water bath and pour plating technique was followed (Surendran *et al*, 2006). 1ml of each sample was pipetted and pour-plated. Plates were allowed to set, inverted and incubated at 37^oC for 18 – 24 hrs. The plates were examined for *Enterobacteriaceae* (Coliforms, *Shigella, Salmonella)* colonies. The *Vibrio cholera* was detected by taking 25ml of the sample blended with 225ml Alkaline Peptone Water (APW), transferred aseptically to a sterile 500ml conical flask and incubated at 36 ± 1^oC. After 6 – 8 hrs and 16 – 24 hrs of incubation, a loopful of culture was taken from the surface growth (pellicle) on to pre-set Thiosulphate Citrate Bile salt Sucrose (TCBS) agar. It was incubated at 36 ± 1^oC for 17 – 24 hrs. After incubation the plates were examined for typical *V. cholera* colonies.

RESULTS AND DISCUSSION

Total Plate Count

The total plate count was highest at RKB – II (4,250 cfu/ml) and lowest in Aqua sports complex (290 cfu/ml). At Rushikonda beach the it was 1080 cfu/ml followed by RKB – I (1000 cfu/ml) and at Tenneti park it was 710 cfu/ml (Fig. 2).

Fig. 1 Sampling stations

Most Probable Number (MPN)

The most probable number (MPN) for each sample for total coliforms, confirmed coliforms and coliforms at RKB – I was 160, at RKB – II (180+), at Rushikonda beach (180+), at Tenneti Park (160) and at Aqua Sports complex it was zero.

Table 1 MPN table for Total Coliforms, Confirmed Coliforms and Fecal Coliforms at sampling stations.

Sampling Stations	Total Coliforms	Confirmed Coliforms	Fecal Coliforms
Rama Krishna Beach (RKB -I)	1:5:4 160	1:5:4 160	1:5:4 160
Rama Krishna Beach (RKB -II)	1:5:4 180+	1:5:4 180+	1:5:4 180+
Aqua sports Complex (ASC)	0	0	0
Tenneti Park (TP)	1:5:4 160	1:5:4 160	1:5:4 160
Rushikonda Beach (RuKB)	1:5:5 160	1:5:5 160	1:5:5 160

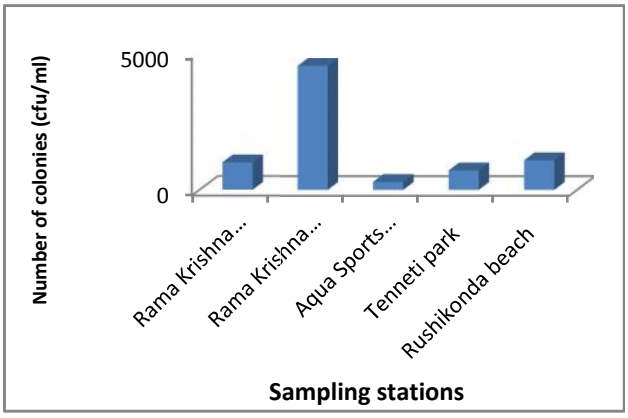

Fig. 1 Sampling stations

Fig. 2 Total plate count at different sampling stations

The *E. coli* was confirmed by Gram's staining as gram negative rods or bacilli. Isolated colonies with greenish metallic sheen and dark purple centered colonies were observed (Fig. 3). *E. coli* was positive to indole and methyl red tests and negative to Voges – Proskauer test and citrate utilization tests.

Enterobacteriaceae

Small (2 – 4 mm diameter) red colonies were observed on the Violet Red Bile Glucose Agar (VRBGA) (Fig.4). The highest colonies (398 cfu/ml) were observed at Rushikonda beach waters and lowest (69 cfu/ml) at Tenneti Park. No colonies were observed at swimming pool water (Table 2).

Faecal Streptococci

Red to pink colonies were observed on KF agar (Fig. 5). The highest colonies (222 cfu/ml) were observed at Rushikonda beach waters and lowest (32 cfu/ml) at RKB- I. No colonies were observed at swimming pool water (Table 2).

Table 2 *Enterobacteriacea* and fecal *Streptococci* at different sampling stations.

	Colonies (cfu/ml)				
Indicator organisms	RKB-I	RKB-II	RuKB	TP	SP
Enterobacteriaceae	80	167	398	69	Absent
Fecal Streptococci	32	120	222	56	Absent

Vibrio cholera No specific colonies of *V. cholerae* was observed in all the water samples.

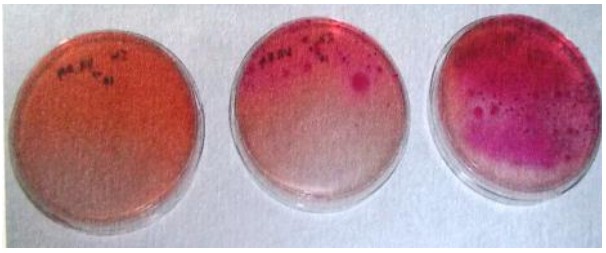

Fig. 4 Small red colonies of *Enterobacteriaceae* on VRBGA medium

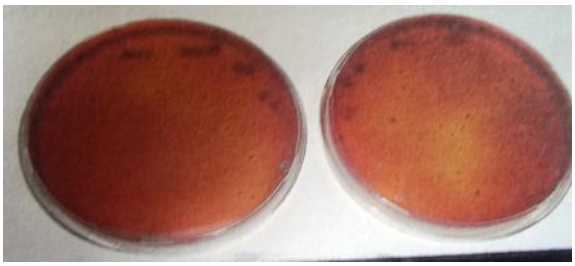

Fig. 5 Red to pink colonies of fecal streptococci on KF agar

The number of colonies of *Enterobacteriaceae* were high in all the water samples when compared with that of the fecal *Streptococci.* Aqua Sports Complex water was completely free from bacteria probably due to the regular hygienic measures taken. *Vibrio cholera* was absent in beach water. The MPN values of coliforms /100ml of water RKB -I & Tenneti Park were lower than RKB -II & Rushikonda beach. The maximum admissible level is <1 indicating very high concentration of bacteria. This may be due to the regular release of domestic sewage into the sea water. Total Plate Count was high at RKB – II followed by Rushikonda beach waters. The samples sites may be highly contaminated with the domestic sewage released into it and hence the low quality of the beach waters. The quantity of bacteria in RKB- I & TP was less when compared to the other beaches. Aqua sports complex water was totally free of bacteria. This may be due to the regular cleaning and chlorination. The water sample collected from the Tenneti Park was found to be better when compared with others. RKB- II sample was of less quality in terms of bacterial loads. The MPN value of RKB-II and Rushikonda beach considered to be high and not advisable to swim.

Clark Turner *et al* (1985) have observed the beach waters of Visakhapatnam are hygienically poor and due to high Pollution Index (PI), Lawson's Bay waters are unsuitable for non-contact recreation. Bordalo (1994) has recorded faecal contamination of the beach waters of two urban coastal beaches, Ourigo and Pastoras. Fuhrman *et al* (1999) have found faecal Coliforms in California and Avalon Bay in South California due to the leaking drains, bird droppings and runoff street water. *Vibrio* bacteria have been identified by Ifremer and Jiang (2000) in the temperate and tropical regions of France which contaminated the sea foods causing gastrointestinal and respiratory illness in the people. Jiang (2001) has also found the incidence of *Vibrio* being influenced by water salinity. Namaihira *et al* (2003) have evaluated the poor water quality of Huayamilpas due to the occurrence of total coliforms, faecal coliforms and faecal *Streptococci* because sporadic drainage of the domestic waste water. Mc Bride *et al* (2005) have found the illness of gastrointestinal and respiratory tracts of tourists and swimmers due to *E. coli.* in the beaches of New Zealand.

Sugumar *et al* (2008) have found that the total coliform bacteria, coliform bacteria and *E. coli* from undetectable to the maximum detectable level of over MPN 140 throughout the year with no seasonal variation in the coastal waters of Thoothukudi and represents a potential risk to public for recreational and fishing activities, because of high *Streptococci* distribution. According to Ajit Kumar *et al* (2009) a positive correlation between biological oxygen demand (BOD) and pathogenic microorganisms and a negative correlation with salinity indicating dominant influence of human activities along the coastal waters of Orissa. Rajagopal *et al* (2013) have observed varied total heterotrophic bacterial population density water from 43 $X10^5$CFU/ml to 182 $X10^5$ CFU/ml and in sediment, it is from 79 $X10^5$ CFU/mg to 259 $X10^4$ CFU/mg at Havelock Island, the Andamans.

Brilliant *et al* (2013) have observed >2400 MPN/100 ml Coliforms and 210 to >2400 MPN/100 ml *Streptococci* during both the pre tourism and post tourism seasons at Varkala Beach. Ramesh Babu *et al* (2014) have reported more enteric bacteria in fishing harbour waters of Visakhapatnam because of indiscriminate discharge of the sewage, industrial effluents and fishing activities, moderate occurrence at Hindustan shipyard and relatively low levels at offshore station (Bhimili). Ranjith and Nagamurugan (2014) have observed the highest frequency of susceptible strains in *Vibrio* sp than *E. coli* of Nagappatinam coastal sediments. According to Maloo *et al* (2014), the indiscriminate use of antibiotics in aquaculture, poultry and other livestock may pose high ecological risk leading to the occurrence of multiple antibiotic resistant bacteria in coastal waters of the Veraval. Sunil Borade *et al* (2015) have found a strong correlation between bacteria with physico-chemical parameters at Veraval coast. Viswanadham and Kondalarao (2015) have observed high concentration of *Vibrio* bacteria (4to1.53x10^3cfu/ml) before Hudhud cyclone and less concentration (3to 1.32x10^3cfu/ml) after the cyclone in the near shore waters of Visakhapatnam.

CONCLUSION

Beach waters are generally used for recreation and swimming activities. Untreated waters due to urbanization, industrialization and other commercial activities, when released increase the bacterial loads that are detrimental to the ecosystem and to human health and welfare.

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