Studies on the Occurrence of Enteric Bacteria in the Estuarine Waters Along the Mangalore Coast

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Occurrence of enteric bacteria in water, sediment and shellfishes of Mulki, Pavanje, Gurpur and Netravathi estuaries of the Mangalore coast is reported. 70 water samples, 71 sediment samples and 37 shellfish samples were analysed in 18 months. Total bacterial load in sediment and shellfishes was found to be more than that in water samples. The total bacterial load was not very high. However, enterococci, particularly coliforms in sediments, water and shellfishes were found to be quite high, indicative of faecal pollution. The incidence of *Salmonella* spp. was recorded in all the estuaries except the Mulki Estuary.

The bacteriology of estuarine waters as well as that of the estuarine fisheries is receiving greater attention in recent times, in view of the higher incidence of pathogenic microorganisms, like *Vibrio parahaemolyticus* and food poisoning organisms like *Clostridium* spp., *Streptococcus faecalis* etc. found in shellfishes collected from these waters. Mangalore is one of the major fishing ports of India and many rivers join the sea all along its coast; as such estuarine fishing, particularly of shellfishes, forms a major activity of this coast. Annually more than 700 t of shellfishes are caught along this coast.

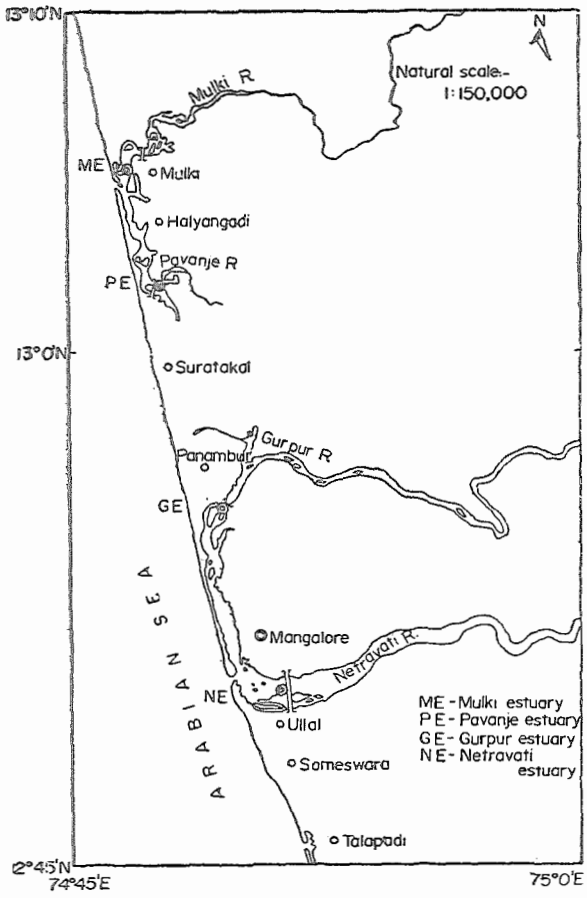

Fig. 1 The sampling sites
ME–Mulky estuary; GE–Gurupur estuary
PE–Pavanje estuary; NE–Netravathy estuary

Bacteriological studies of these estuarine waters are very limited. Incidence of *E. coli* in marine fish and mussels caught from estuaries near Malpe has been reported by Stephen *et al.* (1975). In the present study an attempt has been made to study the occurrence of enterococci, coliforms, *E. coli* and *Salmonella* in sediment, water and shellfishes of four major estuaries of this coast, over a period of eighteen months. The sampling sites are shown in Fig. 1.

Materials and Methods

Water samples were collected in sterilised 250 ml. sampling bottles from different regions of the estuary. The bottom sediment was collected in sterile petri dishes and the shellfishes (mostly clams but a few samples consisted of different species of fish) were collected in sterile plastic buckets. All the samples were stored in insulated containers in ice, brought to the laboratory and analysed the same day. The shells of clam were opened by cutting the adductor muscle with a sterilised blade and 10 g of

composite muscle samples were transferred to homogeniser cups containing 90 ml of sterile physiological saline. Monthly sampling was done throughout the year, except during the monsoon (June to August) from four sites of the Nethravathi, the Gurpur, the Pavanje and the Mulki estuaries. A total of 70 water samples, 71 sediment samples and 37 shellfish samples were collected and analysed over a period of 18 months (September 1979 to February 1981).

For total viable counts, nutrient agar medium was employed using pour-plate technique and the counts were taken after 48 h of incubation at room temperature (28–32°C). To determine enterococci, maltose-azide-tetrazolium chloride agar, for coliforms, desoxycholate agar, for *E. coli,* Tergitol 7 agar, Eosine-methylene blue and violet red bile agar were employed. For detecting *Salmonella,* the samples were put in lactose and tetrathionate enrichment broths respectively and after incubating at 37°C for 24 to 48 h they were streak plated on bismuth sulphite agar. The black colonies appearing on this medium were transferred to TSI agar slants, apart from conducting various biochemical tests for confirmation as described by Harrigan & MeCance (1976). For all these cultures, the incubation temperature was 37°C and the period of incubation was from 24 to 48 h.

For enumeration of coliforms and enterococci from sediment, water and shellfish samples, the most probable number (MPN) method was employed according to standard methods for the examination of water and waste waters (APHA 1976) and as detailed by Harrigan & McCance (1976).

Results and Discussion

Results of the physico-chemical analysis of water and sediment are shown in Table 1. Mulki and Netravathi estuaries showed high range of salinity when compared to the other two estuaries. While the Pavanje Estuary showed a fair amount of salinity and alkaline pH, the Gurpur Estuary showed negligible salinity. The variations of temperature was not significant. Whenever the water column was shallow, higher temperature was recorded in summer. The high pH and salinity in the Mulki Estuary and high salinity in the Netravathi Estuary were probably due to the proximity of the sampling points to the sea, which were about half and one kilometer respectively. While in the other two estuaries sampling points were more than 5 km and showed low pH and salinity.

The data on bacteriological analyses during the first year (September 1979 to July 1980) are shown in Table 2. Sediment and shellfish samples from the Netravathi Estuary showed the highest total plate count (TPC), as compared to sediment samples from other estuaries, which were in the range of 10^3 to 10^5

Table 1. *Results of the physico-chemical analysis of the four estuaries*

Area	Samples analysed	pH	Range, Temp, °C	Salinity, ‰
Mulky estuary	Sediment	7.0–8.0	23.0–27.0	8.5–21.2
	Water	6.9–7.8	20.5–28.0	
Gurpur estuary	Sediment	6.5–7.0	26.5–28.0	0.35–3.0
	Water	6.5–6.8	26.0–30.5	
Pavanje estuary	Sediment	6.7–7.5	27.0–30.5	0.35–6.2
	Water	6.6–7.3	26.0–30.5	
Netravathi estuary	Sediment	6.8–7.0	26.0–27.0	0.4–29.32
	Water	6.3–6.8	26.0–26.5	

Table 2. *Bacteriological analysis of samples from four estuaries for the period September 1979 to July 1980*

Area	Samples analysed	Total no. of samples	TPC/g or ml (mean values)	Nos/g or ml (mean values) Entero-cocci	Coli-forms	*E. Coli*	*Salmonella* sp. % incidence
Mulky estuary	Sediment	12	1.04×10^5 (1.21×10^3 - 3.7×105^5)	Nil	100	2	Nil
	Water	12	2.07×10^2 (1.0×10^1 - 5.6×10^2)	Nil	20	Nil	Nil
	Shellfiah	8	3.08×10^4 (3.0×10^3 - 5.6×10^4)	Nil	400	Nil	Nil
Gurpur estuary	Sediment	14	8.2×10^3 (1.02×10^2 - 2.3×10^4)	400	190	30	7
	Water	14	6.74×10^2 (5.3×10^1 - 2.2×10^3)	22	24	3	Nil
	Shellfish	7	4.75×10^4 (9.5×10^2 - 1.03×10^5)	200	390	40	Nil
Pavanje estuary	Sediment	12	1.53×10^5 (1.3×10^3 - 3.9×10^5)	55	190	35	8
	Water	11	1.80×10^3 (0.1×10^1 - 4.02×10^3)	1	18	9	Nil
	Shellfish	5	8.70×10^4 (2.85×10^2 - 2.56×10^5)	1300	300	30	Nil
Netravathi estuary	Sediment	9	1.34×10^6 (1.9×10^3 - 5.2×10^6)	40	480	40	11
	Water	9	6.20×10^3 (5.1×10^1 - 1.97×10^4)	3	81	4	Nil
	Shellfish	5	5.87×10^5 (2.8×10^3 - 1.21×10^6)	20	900	200	Nil

Figures within brackets indicate range of bacterial load

log. However, bacterial load in the water samples of all the estuaries was comparatively low and of the order of 10^2 to 10^3 logs. While inflow of sewage to all these estuaries was a common feature, the distance from the sea, at which the sewage entered the rivers, varied widely from half a kilometer in the Mulki Estuary, to more than two kilometers in the Pavanje and Gurpur estuaries. The Netravathi on the other hand received the sewage at a distance of about one kilometer from the sea and the sampling was done almost in the same area. This probably accounts for the high TPC counts noted in the sediment and shellfish samples of this estuary. On this account Mulky Estuary also should have shown higher incidence of TPC in sediment and shellfish samples but fairly lower counts were recorded. The river mounts of both the Netravathi and the Mulki estuaries are equally wide but the depth of the water column is more shallow (60–90 cm) in Netravati. But in Mulky Estuary this depth was around 2.4 m. Therefore the dilution effect appears to be the reason for the fairly low counts recorded in samples collected from the Mulki Estuary.

In general, the total bacterial load for sediment, water and shellfish samples from all the four estuaries does not appear to be very high. Erkenbrecher (1981) reported an average bacterial load of 566 x 10^2 and a range of 1–24.00 x 10 per ml of water for the Lynnhaven Estuary and an average load of 1,525 x 10^2/g (dry wt) of sediment from the same estuary. Shellfish (clams) being bottom dwellers and filter feeders, the higher TPC counts than the surrounding waters appears to be understandable. Geldreich & Clarke (1966) demonstrated that bottom feeding fish have higher levels of coliforms, faecal coliforms and faecal streptococci than surface-feeding and predatory fish.

The load of enterococci and particularly coliforms in shellfish and sediment samples from all the four estuaries appears to be very high (Table 2), though the water samples showed lesser counts. For want of bacteriological standards for estuarine waters, it is difficult to comment on these figures. Considering the bacteriological standards for recreational waters in America (total coliforms 1000/100 ml and faecal coliforms 70/100 ml), these figures appear to be very high. However, presence of coliforms to the extent of 235.6/g to more than 400/g and *E. coli* to the extent of 52–64/g in raw cockles have been reported by Ayres (1979). Erkenbrecher (1981) reported a total coliform load of 14–2 40,000 per 100 ml of water and 1–8 13,001/g (dry wt) of sediment samples obtained from the Lynnhaven Estuary and concluded that the estuary is faecally polluted and the bacterial pollution is well above the safe maximum limits for shellfish growing waters.

Similarly, the counts for *E. coli* in the sediment and shelfish samples of all the estuaries, except the Mulki Estuary, are on the higher side and so are the counts for water samples. The incidence of *Salmonella* was recorded in all the estuaries except in the Mulki Estuary. Though the occurrence varied from 7–11% in sediment it should be considered as significant, particularly for shellfish growing areas.

Enumeration of enterococci and coliforms by the MPN method and total plate counts during September 1980 to February 1981 is shown in Table 3. There is reduction in the total bacterial load in all the four estuaries compared to the previous year but the general trend of higher counts in the sediment and shellfish compared to that in water, could be seen during this year also. Higher incidence of enterococci and particularly coliforms was quite evident and fully substantiate the data of the previous year for all the estuaries. One major difference as compared to last year's data is that even water samples from all the four estuaries show much higher counts of coliforms and almost equal to that of shellfish and sediment samples. Although higher incidence of coliforms was noted in the previous year also, such a difference could be due to rainfall, subsequent runoff, seasonal fluctuations of salinity, inflow of sewage, dissolved oxygen, temperature, suspended solids, etc apart from the method of estimation. The differences in coliform densities of sediment, shellfish and water samples, individually and among the four estuaries are highly fluctuating but nevertheless very high for shellfish growing.

Since estuarine fishing and shellfish collection form a major activity in the Mangalore

Table 3. *Bacteriological analyses of samples from four estuaries for the period September* 1980 *to February* 1981

Area	Samples analysed	Total no. of samples	TPC nos/g or ml	MPN per 100 ml (range) Enterococci	Coliforms
Mulky estuary	Sediment	6	3.06×10^2	104–170	170–20000
	Water	6	2.53×10^2	0–275	10700–24000
	Shellfish	3	3.0×10^3	20–94	61–24000
Gurpur estuary	Sediment	6	9.07×10^3	270–575	1100–13200
	Water	6	8.40×10^1	145–305	2450–13200
	Shellfish	3	5.30×10^3	40–9200	11000–24000
Pavanje estuary	Sediment	6	1.62×10^4	150–1910	8075–24000
	Water	6	2.09×10^2	65–869	163–14700
	Shellfish	3	5.6×10^3	83–320	3000–24000
Netravathy estuary	Sediment	6	5.4×10^3	100–370	2500–25000
	Water	6	1.3×10^3	285–950	3000–24000
	Shellfish	6	1.18×10^4	130–640	2400–16000

coast, the present study points out that these estuarine waters are faecally polluted and the pollution is well above the safe maximum limit for shellfish growing.

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