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Prevalence of diarrhegenic serotypes of *Escherichia coli* in the Cochin estuary, along west coast of India

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Prevalence of indicators of faecal pollution such as faecal coliform and faecal streptococci were studied at the Cochin estuary. There was consistently high load of faecal indicator bacteria at all stations. Serotyping of *E. coli* revealed the presence of diarrhegenic serotypes of *E. coli* such as enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC) and uropathogenic *E. coli* (UPEC). The diversity of *E. coli* serotypes was particularly high as we were able to detect more than 40 serotypes. The *E. coli* serotypes also showed spatial and temporal variation. Characterisation of faecal streptococci revealed predominance of *Streptococcus faecalis* subspecies *liquefaciens* followed by enterococcus group and *Streptococcus bovis*. Seasonal variation in the prevalence levels of these organisms showed a higher load of the indicator organisms during the monsoon season, especially that of faecal streptococci indicating a higher land run-off during this period. The results of the present investigations reveal the high degree of faecal pollution in the Cochin estuary posing health hazard to those who use this system for fishing and recreation.

[**Key words**: *Escherichia coli*, faecal pollution, estuary, diarrhegenic serotypes]

Introduction

Population explosion and inadequate infrastructure to properly treat and dispose the sewage has resulted in the discharge of considerable quantities of untreated wastes into natural waters. Sewage effluents contain a wide range of pathogenic microorganisms that may pose a health-hazard to human populations when they are discharged into recreational waters[1]. The increased pollution of the natural waters in conjunction with the improved screening techniques has resulted in the detection of emerging pathogens in areas affected by sewage discharges[2], with subsequent hazard to public health[3]. The presence of indicator organisms in natural waters indicates pollution, but their absence does not necessarily guarantee quality of the water[4].

Cochin estuary, a typical tropical estuary, had undergone considerable pollution in the last decade resulting mainly from the development of satellite townships all across the estuary. The pollution of the estuary is mainly of microbial, as the industrial development had declined in this region. The only industry which contributes to microbial pollution of Cochin estuary is seafood industry, as the wastewater from many factories, which is rich in organic matter, end up in the estuary. Apart from two previous studies[5,6], no systematic studies have been carried out on the microbial pollution of this important natural water body during the last decade. The present study has been taken up to study systematically the pollution status of the Cochin estuary with special reference to indicator bacteria such as faecal streptococci and *Escherichia coli* for a period of one year. Prevalence of diarrhegenic serotypes of *E. coli* has been analysed by serotyping the strains. Seasonal variation in the prevalence level of these organisms as well as the correlation between the indicator and the definite pathogen has also been worked out.

Materials and Methods

The water samples were collected monthly from five different stations (Fig.1) along the Cochin estuary during November 2001–October 2002. The stations were selected based on the closeness to satellite townships and waste input. Two of the stations (Chittoor and Thevara) were fixed upstream, two in the central part of the estuary namely Bolghatty and Marine Science Jetty, and one at the barmouth. Water samples were collected between 7 and 9 am in sterile

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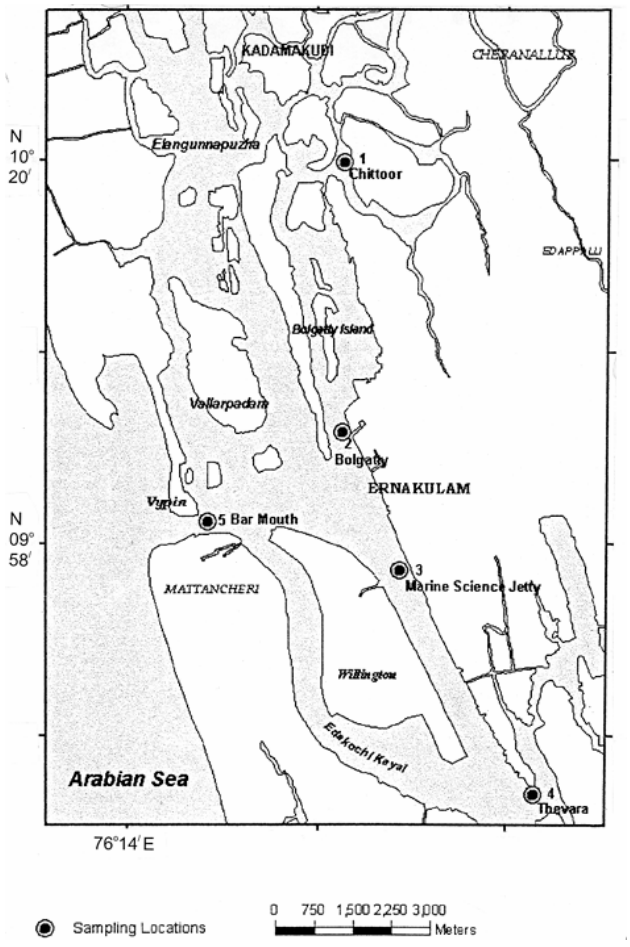

Fig. 1—Map showing sampling stations.

plastic bottles (Tarson, India) one foot below the surface to get a better representation of the water column. Water samples were transported to the laboratory in an icebox and subjected to bacteriological examination within 4 hours of collection. The samples were processed for microbial parameters[7] such as faecal coliform (FC), and faecal streptococci (FS).

A three-tube MPN technique was used to estimate faecal coliforms using E.C. broth (Hi-media) as the medium and incubation at 44.5°C. Inocula from tubes showing growth and gas production were streaked on Eosine Methylene Blue (EMB) agar for the isolation of *E. coli* and incubated at 37°C. Typical *E. coli* like colonies were selected, restreaked to ensure purity and confirmed by indole, methyl red, voges-proskauer and citrate (IMViC) test. Similarly, MPN of faecal streptococci were determined by inoculating azide dextrose broth and incubation at 37°C. Inocula from tubes showing growth were streaked on Pfizer selective enterococcus agar and incubated at 37°C. Typical colonies were isolated, purified and characterised biochemically into different groups. Confirmed *E. coli* cultures were serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh (India).

Results and Discussion

The prevalence of faecal indicator bacteria such as faecal coliforms and faecal streptococci at different stations during the period November 2001 to October 2002 is represented in Table 1. The results revealed that there were considerably high levels of FS and FC at all stations. The values were higher than those recorded near the mouth region of Vembanad lake[5], of which Cochin estuary is a part. The values were also greater than those recorded in Mandovi and Zuary estuarine complex of Goa[8]. The higher load is possible, as the human population has grown considerably in the last two decades and hence the waste generation. Though sewage collection systems are there, many illegal sewers are entering the estuary, especially from market places. Also there is a practice of dumping animal carcasses into the estuary at various points. There are no effective measures to control such activities, which can result in gross contamination of the system, once the waste input goes beyond the critical load of self-purifying capacity of the system.

The mean value of the faecal indicator bacteria for the entire year revealed that station very close to the Cochin City, station 3, had maximum FC load. The load was minimum, though well above the prescribed limits, at upstream station 1. This again indicates the population pressure on infrastructure and resultant increased waste discharge into the system. The mean FC level for the year at station 5 showed that considerable removal of FC is taking place as they reach the Barmouth, which is almost 4 kilometers away from the stations near to Cochin city and the salinity varied between 5-20 ppt during the study period. However, the mean values for the FS level showed an interesting pattern. The highest mean load was recorded at stations 3 and 5 indicating better survival of these bacteria than FC at higher salinities and chemically dynamic nature of the Barmouth region. FC values vary considerably between these two stations and the load was reduced considerably at Barmouth. The mean FS levels were comparatively lower at the upstream station 1 as in the case of FC.

The study period has been categorised into three distinct seasons, such as premonsoon (February–May), monsoon (June–September) and postmonsoon

Table 1—Prevalence of faecal coliforms and faecal streptococci at different stations during Nov. 2001 to Oct. 2002

Sta. No.	Test organism	MPN value (per 100 ml) of FC and FS during the study period											
		Nov. 2001	Dec.	Jan. 2002	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep	Oct.
1	FC[a]	1100	1100	1100	1100	1100	1100	11000	110000	4600	9300	2300	46000
	FS[b]	1100	1100	1100	1100	1100	140	11000	110000	15000	9300	110000	46000
2	FC	1100	1100	1100	1100	1100	1100	4600	110000	15000	110000	4300	4300
	FS	1100	1100	1100	1100	1100	70	11000	110000	110000	24000	110000	110000
3	FC	1100	240	1100	1100	1100	1100	11000	110000	110000	110000	21000	21000
	FS	1100	1100	1100	1100	1100	930	11000	110000	110000	46000	110000	110000
4	FC	1100	120	1100	1100	1100	1100	11000	110000	9300	110000	900	900
	FS	1100	1100	1100	1100	1100	4600	11000	110000	110000	24000	21000	15000
5	FC	1100	23	1100	1100	1100	1100	11000	110000	46000	9300	9300	15000
	FS	1100	1100	1100	1100	1100	2100	11000	110000	110000	46000	110000	110000

[a]Faecal coliform, [b]Faecal Streptococci

Table 2—Seasonal variation of indicator bacteria at different stations during Nov. 2001 to Oct. 2002

Station no.	Premonsoon		Monsoon		Postmonsoon	
	FC[a]	FS[b]	FC	FS	FC	FS
1	3575	3335	14580	35360	1100	1100
2	1975	3317	28920	73000	1100	1100
3	3575	3532	54600	77400	813	1100
4	3575	4450	26420	36200	773	1100
5	3575	3825	18120	77400	741	1100

Premonsoon – Feb. to May; Monsoon – Jun. to Sep., Postmonsoon – Oct. to Jan.
[a]Faecal coliforms (Values are indicative of mean MPN values/100ml for the season)
[b]Faecal streptococci (Values are indicative of mean MPN values/100ml for the season)

Table 3—Different serotypes of *Escherichia coli* and different groups of faecal streptococci encountered in the Cochin estuary during Nov. 2001 to Oct. 2002

E. coli serotypes (n=81)	% incidence	*E. coli* serotypes (n=81)	% incidence	Faecal streptococci (n=72)	% incidence
O1	4.9	O63	2.5	*Streptococcus faecalis* Sub sp. *Liquifaciens*	65.3
O39	2.5	O19	1.2	*Streptococcus bovis*	6.7
O173	1.2	O51	2.5	*Enterococcus* sp.	10.7
O78	1.2	O139	2.5	Unidentified	17.3
O157**	3.7	O86	1.2		
O8*	2.5	O116	1.2		
O101	7.4	O150	1.2		
O91	1.2	O32	1.2		
O165	2.5	O135	1.2		
O106	1.2	O102	1.2		
O22	4.9	O15	1.2		
O104	1.2	O29	1.2		
O107	1.2	O9	3.7		
O25	8.6	O88	2.5		
O14	1.2	O80	1.2		
O69	2.5	O131	1.2		
O117	1.2	O66	1.2		
O105	1.2	O30	1.2		
O156	3.7	O20***	1.2		
O113	1.2	$O2^{+}$	1.2		
O60	1.2	UT	3.7		
O33	7.4				

*Enterotoxigenic *E. coli* (ETEC). **Enterohemorrhagic *E. coli* (EHEC)
***Enteropathogenic *E. coli* (EPEC), [+]Uropathogenic *E. coli* (UPEC), UT – Untypable.

(October–January), which is a marked feature of the weather in the study area. The mean value for FS and FC during each season for each station has been presented in Table. 2. The results indicated substantially high level of indicator bacteria, both FS and FC, at all stations during monsoon months. The samples collected from stations 3 had the maximum of both FS and FC, though only of FS high at station 5. The high load during the monsoon months is anticipated as the heavy monsoon showers bring in heavy land run-off into the Cochin estuary, resulting in increased levels of waste discharge into the system. Also the inactivating effect of major self-purifying factor such as sunlight[9] is very much reduced during the monsoon months as the sky is almost always overcast.

Another important observation made during the seasonal studies is the significant variation in the FS and FC levels during the monsoon months, while there was no significant variation between the mean prevalence of these organisms during the premonsoon and postmonsoon periods. FS levels were considerably higher than FC at all stations during the monsoon months, which is indicative of the huge land run off into this receiving system. The high load of FS is most likely to be from the faecal environment of other domesticated animals, which reaches the system during the monsoon months.

The faecal coliform bacteria were characterized biochemically to see the prevalence of *E. coli*. *Escherichia coli* was isolated consistently from all stations. Strains of *E. coli* were serotyped at National *Salmonella* and *Escherichia* Centre (Kasauli, Himachal Pradesh, India) to see the diversity. The results (Table 3) revealed remarkable diversity of the *E. coli* strains in the system. More than 40 different serotypes were encountered which included potential pathogens such as enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and uropathogenic *E. coli* (UPEC). The EHEC levels were considerably higher and this is for the first time the isolation of this emerging pathogen is being reported from the Cochin estuary. The interesting observation was that EHEC was isolated from the station nearest to Cochin city, whereas none of the other stations gave any positive isolation for this organism during study period. This is suggestive of the possibility of release of this organism through hospital waste from many of the hospitals in and around Cochin city. Though prevalence of faecal indicator bacteria from Vembanad lake has been reported earlier, serological characterization and existence of different serotypes were not reported so far.

Faecal streptococci were also subjected to biochemical characterization in order to group them into different species. The results (Table 3) indicated the predominance of *Streptococcus faecalis* sub sp. *liquefaciens*, followed by enterococcus group and *Streptococcus bovis*. Except for the monsoon months, the FC levels were greater than FS indicating human source of pollution of the study environment. This is again reconfirmed by the diversity of the *E. coli* serotypes in the system, which showed great diversity.

In conclusion, the results of the present study are indicative of considerable microbial pollution in the Cochin estuary. Emerging pathogens such as EHEC, ETEC and EPEC are thriving in the system, which is a potential danger to the valuable fish and shellfish resources of this estuary as well as those who use it for recreation. Coastal waters are being used for several purposes such as domestic, industrial and for aquaculture. The results revealed that monitoring of faecal indicator bacteria and pathogens in coastal and estuarine waters could be made mandatory for impact assessment studies while these resources are used for the above-mentioned purposes.

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