

Occurrence and seasonal variation of bacterial indicators of faecal pollution along Thoothukudi coast, Tamil Nadu

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Abstract: The prevalence and seasonal variation of bacterial indicators of faecal pollution such as total coliform bacteria, faecal coliform bacteria, *Escherichia coli* and faecal streptococci were investigated in samples of water and beach sand from the four fish landing centres of Thoothukudi. Further, the samples were screened for *Salmonella* to study the reliability of faecal indicator bacteria as an index of human pathogenic bacteria. Total coliform bacteria, faecal coliform bacteria and *Escherichia coli* were isolated from all four landing centres from undetectable to the maximum detectable level of over Most Probable Number 140 throughout the year with no obvious seasonal variation. Faecal streptococci were also detected in most samples. There was no discernable relationship between faecal indicators and physical parameters. The relationship between faecal indicators and *Salmonella* was not significant ($p > 0.05$). The results showed that the coastal waters along Thoothukudi is polluted and presents a potential risk to public for recreational and fishing activities.

Key words: Faecal pollution, Bacterial indicators, *Escherichia coli*, *Salmonella*, Fish landing centres
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Introduction

Tremendous increase in human activities, urbanization and industrialization has disturbed the balance of the coastal environment. The coastal waters are contaminated due to human activity, dumping of waste and discharge of domestic sewage into the coastal environment. All these could contaminate the coastal waters and render the beach unsuitable for recreational and fishing activities. Therefore, it is necessary to understand the magnitude of faecal pollution in the environment. A survey of indicator bacteria such as total coliform bacteria, faecal coliform bacteria, *Escherichia coli* and faecal streptococci will throw considerable light on the sanitary condition of water and serve as guidelines for recreational and fisheries related activities (Bitton, 1994; Krishnan *et al.*, 2007). The extent of faecal pollution increases during monsoon and post-monsoon (Raveendran *et al.*, 1978; Gore *et al.*, 1979) is mainly due to land drainage. Further, environmental factors like salinity, temperature, turbidity, pH, tides *etc.*, condition the persistence of faecal pollution in water (Serrano *et al.*, 1998). Presence of faecal indicator organisms in water and beach sand samples have been reported in several beaches along the Indian coasts (Raveendran *et al.*, 1978; Gore *et al.*, 1979; Vaidya *et al.*, 2001; Nallathambi *et al.*, 2002). Among the indicator organisms, faecal coliforms and *E. coli* were reported to be predominant over faecal streptococci in Bhavnagar coast (Vaidya *et al.*, 2001). *Salmonella* has been isolated from coastal waters worldwide including Indian coastal environments (Vaidya *et al.*, 2001; Aulicini *et al.*, 2001; Iyer, 1989). However, the reliability of faecal indicator bacteria to predict the presence of *Salmonella* is still a matter of debate. It is generally agreed that *Salmonella* is present at high densities of indicator organisms, however, isolation of *Salmonella* in the absence of indicators of faecal pollution has also been documented (Polo *et al.*, 1998). The present study was undertaken to study the seasonal

variations of faecal indicator bacteria from water and beach sand samples along Thoothukudi coast and to investigate the reliability of faecal indicator bacteria as an index of *Salmonella*.

Materials and Methods

Water and sand samples were collected at monthly intervals from the four fish landing centres of Thoothukudi namely Thirespuram landing centre, Fishing harbour, South landing centre and Port beach landing centre for one year from June 2003 to May 2004. Samples of sea water were collected from the surf beaten zone in sterilized glass bottles. Sand samples from the intertidal zone were collected in sterilized polythene bags using sterile spatula. Water samples were analyzed for the physico-chemical parameters namely temperature, pH, dissolved oxygen, salinity and ammonia. Temperature of the seawater was measured using an ordinary Celsius mercury thermometer, pH was measured using a pH meter, dissolved oxygen was estimated employing Winkler's method, salinity of sea water was estimated by Harvey's direct titration method and ammonia was determined by indophenol blue method (FAO, 1975).

Water and sand samples were analyzed for total coliform bacteria (TC), faecal coliform bacteria (FC), *E. coli* (EC) and faecal streptococci (FS). Total coliform bacteria, faecal coliform bacteria and *E. coli* were enumerated by three tube Most Probable Number (MPN) technique (Speck, 1976). After 24 hr of incubation at 37°C 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 faecal coliform bacteria after 24 hr of incubation at 44.5 ± 0.5°C. From the positive tubes of EC broth a loopful of culture was streaked on eosine methylene blue agar (EMB) agar and colonies showing metallic sheen were recorded as presumptive *E. coli*. Further, typical colonies



Table - 1: Physical parameters of water samples from the fish landing centres of Thoothukudi

Sampling sites	Temp. (°C)	pH	Salinity (ppt)	DO (ppm)	NH ₃ (µg NH ₃ -N l ⁻¹) (ppm)
Thirespuram landing centre					
Max.	34.00	7.80	37.00	3.62	225.00 (3.15)
Min.	30.00	7.52	30.00	0.45	21.00 (0.294)
Mean	31.60	7.64	33.38	1.78	89.19 (1.25)
Fishing harbour					
Max.	34.00	8.05	35.6	3.90	52.43 (0.734)
Min.	30.00	7.90	32.00	2.68	15.20 (0.213)
Mean	31.80	7.99	35.08	3.15	32.21 (0.451)
South landing centre					
Max.	34.00	8.24	34.50	4.58	71.00 (0.994)
Min.	30.00	7.87	31.00	1.78	7.50 (0.105)
Mean	31.40	8.00	33.46	3.39	38.82 (0.543)
Port beach landing centre					
Max.	33.00	8.04	35.5	5.03	14.60 (0.204)
Min.	29.00	7.91	28.00	3.10	12.40 (0.174)
Mean	30.80	7.98	33.12	4.10	13.50 (0.189)

· µg at NH₃-N l⁻¹ x Atomic weight = mg l⁻¹/1000 = ppm

Table - 2: Occurrence of faecal indicator bacteria and *Salmonella* at Thirespuram landing centre

Month	Water					Sand				
	TC	FC	EC	FS	<i>Salmonella</i>	TC	FC	EC	FS	<i>Salmonella</i>
June 03	> 140	> 140	30	2.63 X 10 ³	A	> 140	> 140	> 140	1.03 X 10 ³	A
July	> 140	> 140	20	9.20 X 10 ²	A	110	110	3.5	1.40 X 10 ³	A
August	> 140	> 140	5.3	1.00 x 10 ²	A	> 140	> 140	30	2.00 x 10 ²	A
September	> 140	> 140	> 140	1.93 X 10 ³	A	110	110	110	2.00 X 10 ²	A
October	> 140	> 140	> 140	3.10 X 10 ²	P	> 140	> 140	> 140	1.00 X 10 ²	P
November	> 140	> 140	> 140	4.90 x 10 ²	A	> 140	> 140	> 140	4.00 x 10 ²	A
December	> 140	> 140	> 140	1.30 X 10 ²	P	> 140	> 140	110	2.00 X 10 ²	P
January 04	110	110	110	2.00 X 10 ¹	A	110	110	1.6	1.00 X 10 ¹	P
February	> 140	> 140	> 140	Est. < 10	A	> 140	> 140	30	9.00 x 10 ²	P
March	> 140	> 140	> 140	1.80 X 10 ²	P	> 140	> 140	> 140	1.40 X 10 ³	A
April	> 140	> 140	5.3	2.40 X 10 ²	P	2.0	1.1	1.1	1.00 X 10 ²	A
May	> 140	> 140	5.3	1.90 x 10 ²	A	> 140	> 140	45	7.00 x 10 ²	A

TC = Total coliform bacteria, FC = Faecal coliform bacteria, EC = *Escherichia coli*, FS = Faecal streptococci, P = Present, A = Absent

TC, FC, EC in MPN ml⁻¹ or g; FS in CFU ml⁻¹ or g⁻¹

on EMB agar were purified and subjected to IMViC tests and confirmed as *E. coli*. Pour plate technique using kenner faecal (KF) agar was employed for the estimation of faecal streptococcal count and typical colonies were counted after incubation at 37°C for 48 hr and expressed as colony forming unit (CFU) per ml or g of the sample. *Salmonella* was confirmed following standard procedures (AOAC, 1998). *Salmonella* was detected by pre enrichment in lactose broth, selective enrichment in tetrathionate broth and selenite cysteine broth followed by selective plating on xylose lysine deoxycolate (XLD) agar and bismuth sulphite agar (BSA). Typical colonies were purified, subjected to biochemical tests and finally confirmed serologically using poly 'O' and poly 'H' antisera (AOAC, 1998).

Analysis of variance (ANOVA) technique was followed to find whether significant differences existed during the study period and the sampling sites for the parameters studied. Pearsons correlation coefficient (*r*) was calculated to find out the relationship between faecal indicators and *Salmonella* (Snedecor and Cochran, 1962).

Results and Discussion

Physical parameters like pH, salinity and temperature did not show greater variation with seasons and sampling sites (Table 1). However, Port beach showed slightly lower temperature than other landing sites. Dissolved oxygen was highest in Port beach and lowest in Thirespuram while ammonia content was highest in Thirespuram and lowest in Port beach. The mean salinity of water in all landing

Table - 3: Occurrence of faecal indicator bacteria and *Salmonella* at Fishing harbour

Month	Water					Sand				
	TC	FC	EC	FS	<i>Salmonella</i>	TC	FC	EC	FS	<i>Salmonella</i>
June 03	45	45	45	1.00 X 10 ¹	A	45	25	25	Est. < 10	A
July	0.9	0.4	0.4	Est. < 10	A	2	0.7	0.3	Est. < 10	A
August	2.5	2.5	0.4	2.00 x 10 ¹	A	7.5	7.5	0.6	Est. < 10	A
September	0.7	0.7	0.7	1.00 x 10 ¹	A	0.9	0.4	0.4	Est. < 10	A
October	1.5	1.5	1.5	Est. < 10	P	1.5	1.5	1.5	Est. < 10	P
November	11	20	3.5	1.00 x 10 ¹	A	20	9.5	9.5	Est. < 10	A
December	4.5	4.5	4.5	Est. < 10	A	4	2.5	2.5	Est. < 10	A
January 04	0.4	0	0	Est. < 10	A	0.4	0.4	0.4	Est. < 10	P
February	0.7	0	0	Est. < 10	A	0.4	0.4	0.4	Est. < 10	A
March	1.5	0.3	0.3	Est. < 10	P	2.5	2.5	2.5	Est. < 10	A
April	> 140	> 140	0.7	1.00 x 10 ²	A	1.6	1.6	0.6	Est. < 10	A
May	3	3	0.3	Est. < 10	A	3	2	0	Est. < 10	P

Abbreviations as in Table 2

Table - 4: Occurrence of faecal indicator bacteria and *Salmonella* at South landing centre

Month	Water					Sand				
	TC	FC	EC	FS	<i>Salmonella</i>	TC	FC	EC	FS	<i>Salmonella</i>
June 03	45	45	25	1.00 X 10 ¹	A	20	20	9.5	Est. < 10	A
July	> 140	> 140	20	5.00 X 10 ²	A	110	110	3.5	2.00 X 10 ²	A
August	> 140	> 140	15	Est. < 10	A	> 140	> 140	> 140	Est. < 10	A
September	> 140	> 140	3.5	1.90 X 10 ²	A	110	110	1.5	1.00 X 10 ²	A
October	> 140	> 140	2	8.20 X 10 ²	A	> 140	> 140	20	1.00 X 10 ²	A
November	> 140	> 140	> 140	4.20 x 10 ²	A	> 140	> 140	> 140	3.00 x 10 ²	A
December	45	45	7.5	1.80 X 10 ²	A	> 140	> 140	110	2.00 X 10 ²	A
January 04	30	30	6.5	4.00 X 10 ²	A	> 140	> 140	15	8.20 X 10 ²	A
February	9.5	4.5	4.5	Est. < 10	A	20	2.5	0.9	3.00 x 10 ²	P
March	0.4	0.4	0	Est. < 10	A	15	9.5	4.5	Est. < 10	A
April	> 140	> 140	25	2.00 X 10 ¹	P	7.5	7.5	7.5	Est. < 10	P
May	110	110	110	1.00 x 10 ¹	P	110	110	3.5	2.00 x 10 ²	A

Abbreviations as in Table 2

centres was 33 parts per thousand (ppt) except in Fishing harbour where the salinity was 35 ppt, being an enclosed bay.

Occurrence of microbial parameters indicating faecal pollution and *Salmonella* in the four fish landing sites along Thoothukudi is presented in Table 2 to 5. Total coliform bacteria were high in water samples of all landing centres and were at the maximum detectable level of over MPN 140 ml⁻¹ at Thirespuram throughout the study. This might be due to the mixing of sewage from Thoothukudi town through the Buckle channel. Similar trend in total coliform bacteria was observed in South landing centre, though the maximum level of MPN > 140 ml⁻¹ was recorded during six months of the study period. This may be due to the settlements nearby which lead to defecation along the beaches. The situation is much better in Fishing harbour which is an enclosed bay, where the total coliforms were maximum during April and minimum during January. The total coliform bacteria were comparatively less in water samples of Port beach, which is more a recreational beach than a landing centre. The counts varied from MPN 0 to 45 ml⁻¹. The total coliform bacteria varied significantly between sampling

sites (p<0.01) and months (p<0.05). The influence of seasons on the occurrence of total coliform bacteria was not well pronounced as reported for Cherai beach, Kerala (Raveendran *et al.*, 1978) and Cochin backwaters (Gore *et al.*, 1979) where their numbers were higher during monsoon and post-monsoon mainly due to land runoff. However, the total coliform bacteria recorded in the present study were higher than those recorded from these beaches. Similar to water samples, sand samples from Thirespuram also harboured high numbers of total coliform bacteria. However, there was a sudden drop in the levels of total coliform bacteria (MPN 2 g⁻¹) at Thirespuram during April although the water had very high levels of bacteria. Significant variation existed among sampling sites (p<0.01) in sand samples, however, the monthly variation was not significant (p>0.05). The total coliform bacteria in sand samples were higher than those reported for Cherai beach (Raveendran *et al.*, 1978), Cochin backwaters (Gore *et al.*, 1979) and Port Blair bay, Andamans (Nallathambi *et al.*, 2002).

Faecal coliform bacteria were detected at the maximum detectable limit in water samples of Thirespuram landing centre,



Table - 5: Occurrence of faecal indicator bacteria and *Salmonella* at Port beach landing centre

Month	Water					Sand				
	TC	FC	EC	FS	<i>Salmonella</i>	TC	FC	EC	FS	<i>Salmonella</i>
June 03	0.4	0.4	0.4	Est. < 10	A	0.4	0.4	0.4	Est. < 10	A
July	1.5	1.5	1.5	Est. < 10	A	0.9	0.4	0.4	Est. < 10	A
August	0.6	0.6	0.6	Est. < 10	A	3	3	0.4	Est. < 10	A
September	0.4	0	0	Est. < 10	A	0.7	0.7	0	Est. < 10	A
October	0	0	0	1.00 x 10 ¹	A	0	0	0	1.00 x 10 ¹	A
November	45	2.5	2.5	Est. < 10	P	2.5	0.9	0.4	Est. < 10	A
December	7.5	7.5	2	2.00 x 10 ¹	A	11.5	11.5	7.5	2.00 x 10 ¹	P
January 04	0.2	0.2	0.2	Est. < 10	A	0.7	0.4	0.4	Est. < 10	P
February	0.9	0.4	0.4	Est. < 10	A	0.3	0	0	Est. < 10	A
March	1.5	0.9	0.9	1.00 x 10 ¹	P	0.9	0	0	Est. < 10	P
April	20	20	9.5	3.00 x 10 ¹	A	4	4	4	Est. < 10	A
May	0.7	0.7	0.7	1.00 x 10 ¹	P	20	20	0.9	Est. < 10	A

Abbreviations as in Table 2

Table - 6: Correlation coefficient (r) between faecal indicator bacteria and *Salmonella*

Month	Water				Sand			
	TC	FC	EC	FS	TC	FC	EC	FS
Thirespuram	0.213*	0.213*	0.249*	-0.335*	0.136*	0.213*	-0.047*	-0.344*
Fishing harbour	-0.235*	-0.198*	-0.142*	-0.127*	-0.267*	-0.273*	-0.149*	0.0*
South landing centre	0.280*	0.280*	0.384*	-0.345*	-0.263*	-0.655*	-0.423*	-0.0711*
Port beach	0.386*	0.175*	-0.052*	0.083*	0.063*	0.051*	0.381*	-0.174*

Abbreviations as in Table 2, *p > 0.05

which was found to be the most polluted sampling site. This was followed by South landing centre, Fishing harbour and Port beach landing centre. High numbers of faecal coliforms during monsoon and post-monsoon months have been reported in Cherai beach, Cochin backwaters, Bhavnagar coast, Port Blair bay, Andamans and Nagore, east coast of India (Goyal *et al.*, 1977) which was due to land runoff. But this phenomenon could not be applied to Thoothukudi coast due to the failure of monsoon during the study period. Samples of sand from Thirespuram landing centre harboured higher faecal coliform bacteria compared to other landing centres where there is no mixing of domestic sewage. Earlier studies from India reported higher concentration of faecal coliform bacteria in sand than in water (Vaidya *et al.*, 2001; Mohandass and Bharathi, 2003). However, in the present study they were lesser in sand compared to water samples. The faecal coliform bacteria from sand samples were higher than those reported from west coast (Srikantaiah *et al.*, 1985) and lower than the values reported from east coast. In water and sand samples, significant variation existed between sampling sites ($p < 0.01$) while monthly variation was not significant ($p > 0.05$). Negative correlation was observed between faecal coliform bacteria and dissolved oxygen ($p < 0.05$) in Thirespuram, Faecal coliform and ammonia ($p < 0.01$) in South landing centre and faecal coliform and pH ($p < 0.05$) in Fishing harbour.

Escherichia coli showed greater variation among samples during different seasons and highest count was recorded in water

samples of Thirespuram landing centre and lowest from Port beach. Thirespuram waters recorded maximum levels of *E. coli* for over six months from September to March and there was no significant relationship with season. Samples of sand were also found to have high levels of bacteria except during January and April. *E. coli* levels were relatively low at Fishing harbour where it varied from MPN 0 to 45 ml⁻¹ in water and from MPN 0.3 to 25 g⁻¹ in sand samples. At South landing centre, the level varied from 0 to > 140 ml⁻¹ in water and 0.9 to > 140 g⁻¹ in sand. At Port beach, the level of *E. coli* was very less both in water and sand samples. Significant variation existed between sampling sites ($p < 0.01$). The present study showed higher *E. coli* number than the report from east coast (Gore *et al.*, 1979) and lesser than those reported from the west coast of India (Vaidya *et al.*, 2001). *E. coli* number was lesser in sand compared to water samples of South landing centre. However, laboratory studies (Gerba and McLeod, 1976) showed longer survival of *E. coli* in sand compared to water due to higher levels of organic content. A significant variation existed among sampling sites ($p < 0.01$) in sand samples and monthly variation was insignificant ($p > 0.05$). Present study recorded lower *E. coli* number than those reported from Bhavnagar coast (Vaidya *et al.*, 2001) and higher than those reported from Cochin coast. A negative correlation existed between *E. coli* and temperature in Thirespuram water ($p < 0.01$) and Fishing harbour ($p < 0.05$), *E. coli* and ammonia in South landing centre ($p < 0.01$). There was also negative correlation between *E. coli* and pH in waters of Thirespuram ($p < 0.05$).

Faecal streptococci showed greater fluctuations in water samples of all four landing centres and could not be detected for more than six months in water samples of Fishing harbour and Port beach landing centre. The counts were higher in Thirespuram indicating higher pollution of the landing centre. Significant variation ($p < 0.01$) existed among sampling sites in water samples. Although faecal streptococci gives only supplementary evidence of faecal pollution, they are still considered better indicators than coliforms because of their inability to grow and multiply in water or virgin soils (Vaidya *et al.*, 2001). Faecal streptococcal count could not be related to seasons contrary to earlier reports where higher counts were recorded during monsoon and post-monsoon months (Raveendran *et al.*, 1978; Gore *et al.*, 1979; Vaidya *et al.*, 2001). Compared to water samples, faecal streptococcal count was lesser in sand samples and could not be detected at Fishing harbour throughout the study. At Port beach also, faecal streptococci were not encountered in sand except in October. Faecal streptococcal count in sand samples was greater than earlier reports from Cherai beach, Kerala, Cochin backwaters and lesser than those reported from Bhavnagar coast.

The relationship between faecal indicator bacteria and human pathogenic bacteria has been extensively studied (Geldreich, 1972; Dutka and Bell, 1973; Goyal *et al.*, 1977; Thomson, 1981; Venkateswaran and Natarajan, 1987). However, the reliability of faecal indicator bacteria to predict the presence of *Salmonella* is inconclusive. Correlation coefficient (r) was worked out for *Salmonella* versus total coliform bacteria, faecal indicator bacteria, *E. coli* and faecal streptococci in both water and sand samples from all four landing centres of Thoothukudi (Table 6). The results could not establish any relationship ($p > 0.05$). *Salmonella* could be isolated in samples of the fish landing centres of Thoothukudi even when the concentration of faecal coliform bacteria was low. Similar observations were recorded by Dutka and Bell (1973) in Botswana, South Africa. On the contrary, Geldreich (1972) could establish a relationship and reported that when the faecal coliform was over MPN 20 ml⁻¹, *Salmonella* was always present in water samples from canal communities along Texas coast (Goyal *et al.*, 1977). However, in this study *Salmonella* was absent in many samples even when the faecal coliform bacteria were over MPN 140 ml⁻¹. Polo *et al.* (1998), documented the presence of *Salmonella* in the absence of faecal indicators as in the present study where *Salmonella* could be isolated in the absence of *E. coli* and faecal streptococci. Although a significant correlation between *Salmonella* and microbial indicators was documented in coastal waters of Spain (Serrano *et al.*, 1998), no such relationship could be established from water samples of estuarine, lake and marine biotypes of Tamil Nadu (Venkateswaran and Natarajan, 1987). Therefore, although it is traditionally assumed that the presence of faecal indicators predict the presence of intestinal pathogens, no clear relationship could be established between *Salmonella* and faecal indicator bacteria ($p > 0.05$) and

concluded that traditional faecal indicator bacteria are of little value as indicators of the possible presence of *Salmonella*.

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