Increased Prevalence of Indicator and Pathogenic Bacteria in the Kumarakom Lake: A Function of Salt Water Regulator in Vembanadu Lake, A Ramsar Site, Along West Coast of India

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ABSTRACT

Prevalence of indicator bacteria such as *Escherichia coli* and pathogenic bacteria like *Vibrio Cholerae*, *Vibrio Parahaemolyticus* and *Salmonella* were analysed in Vembanadu lake (9035'n 76025'e), along the west coast of India for a period of one year (October 2004- 2005 September). Estuarine water samples were taken monthly from ten stations on the southern (inside bund region) and northern sides (outside of the bund) of a salt water regulator constructed in Vembanadu lake. The results indicated that substantially high level of indicator bacteria within the bund region of the lake during monsoon months and at the time of closure of the bund than the northern region. The results highlighted, that the water body is polluted with high faecal coliform bacteria with mean mpn value ranged from 1718-7706/100 ml. *E. Coli*, *V. Cholerae*, *V. Parahaemolyticus* and *Salmonella* serotypes such as *S. Paratyphi* a, b, c and *S. Newport* were consistently isolated from all the station. To our knowledge this is the first report on the isolation of these *Salmonella* serovars from this lake. *E. coli* showed highest percentage of incidence (85.6-86.7%) followed by *Salmonella* (42-57%) and *V. Cholerae* (40-45%) and *V. Parahaemolyticus* (31.5-32%). The increased prevalence of indicator and pathogenic bacteria within the bund region may be due to the altered flow patterns due to the salt water regulator. The results of the present study suggest that the high faecal Coliform density and pathogen concentration in this water body will pose a serious health hazard to those who use this system for fishing and recreation.

Keywords: Escherichia coli, Vibrio Parahemolyticus, Vibrio Cholerae, Salmonella, Vembanadu Lake, India

INTRODUCTION

Environmental pollution, a potential global problem, has rendered waters along the coastline and recreational beaches unsatisfactory for public use. Population explosion and inadequate infrastructure to properly treat and dispose the sewage, lack of sanitary condition, poverty, over exploitation of natural water has resulted in the discharge of considerable quantities of untreated waste into the natural waters. This organic pollution is especially severe in the coastal waters due to the large density of inhabitants in coastal areas (Scialabba 1998). Direct discharge of domestic waste, leaching from poorly maintained septic tanks, and improper management of farm waste are suspected as the major sources of waterborne disease (Huttyp. 1990). Sewage effluent contains a wide range of pathogenic micro-organisms that may pose a health hazard to human population when they are discharged into the recreational waters (Borrego et. al., 1997) and the health hazard could be severe in heavily populated country such as India. In India, almost three-quarters of a billion people live in rural areas without access to safe drinking water and water-borne infections are a major cause of morbidity (Patil et al., 2002). Diseases such as enteric fever and diarrhoeal diseases are highly endemic to India and are major public health problems among the children under the age of five years. The planning commission in its report ‘India Assessment 2002 – water supply and sanitation’ acknowledges that mortality and morbidity levels due to water borne diseases in the country are unacceptably high (www.cseIndia.org/programme/health/pdf/conf2006/a1water.pdf). On a global basis, around 2 million deaths per year are attributed to water-borne diseases especially due to diarrhoea in children (Gordon et al., 2004). Typhoid and paratyphoid fever resulting in an annual incidence of about 17 million cases world wide (Kindhauser 2003), and India has the highest incidence of typhoid, around three million cases each year (anon, typhoid, in The Hindu, February, 23, 2003).

Faecal Coliform, *Escherichia coli* (the predominant member of faecal Coliform group) are an operationally defined grouping of enteric bacteria whose presence in natural waters is used an indicator...
of recent faecal contamination, and therefore, the possible presence for pathogenic Micro organisms (Rhodes and Kator 1998) but their absence does not necessarily guarantee quality of water (Dutka et al., 1973). E. coli includes several pathogenic serotypes such as enterotoxigenic E. coli (ETEC), Enteroinvasive E. Coli (EIEC), enterohemorrhagic e. coli (EHEC), enteropathogenic E. coli (EPEC) Enteroaggregative E. coli (EAGGEC) and Enteroadhesive E. coli (DAEC), which are of public health significance worldwide and are major cause of acute diarrhoea in children in developing countries (Nataro and Kaper 1998; Rodrigues et al., 2002).

The presence of human pathogenic bacteria such as Salmonella, V. cholerae, V. parahaemolyticus, and pathogenic serotypes of E. coli has been reported from coastal areas (Venkateswaran et al., 1989; Daniels et al., 2000; Hatha et al., 2004). Among the aquatic microflora V. cholerae and V. parahaemolyticus are responsible for most infections by Vibrios in developing as well as in developed countries (Faruque et al., 1998). Several food borne outbreaks due to the consumption of shellfish grown in sewage contaminated water has been reported (Daniels et al., 2000). Costal areas often provide very important recreational and economic resources (Costanza et al., 1997) and therefore the trophic status and quality of coastal waters, the safety of shellfish and fish farming waters are concern for many countries including India.

Kumarakom lake is a part of the Vembanadu estuary and lifeline of people around Kumarakom. The population in the study area is scattered in many small islands without any central facility for the effective waste collection and disposal, the Kumarakom lake acts as major sink for all domestic and industrial waste. Also the number of people using the system for agriculture, fishing, transportation and recreation is much more than the other parts of the Vembanadu lake. The availability of pure drinking water is very low in this region and this lake water is being used for different domestic purposes. Water related diseases are very common in these regions even though most of them were not reported officially. The water quality problem of the lake is further compounded by the construction of a salt water exclusion bund which seriously affects the flow patterns of the lake. Though some published data are available on the water quality of cochin region of Vembanadu lake there are virtually no reports available on the microbial pollution from present study area. Hence the present study has been carried out with an objective to systematically examine the prevalence of indicator and pathogenic micro-organisms such as E. coli, V. cholerae, V. parahaemolyticus and Salmonella at various stations outside and inside of salt water regulator installed at the Kumarakom region of Vembanadu Lake.

MATERIALS AND METHODS

Description of study area and sampling

Vembanadu lake, an important Ramsar site, lies 0.6-2.2 m below mean sea level (MSL) along the west coast of India (9°35’N 76°25’E) and have a permanent connection with the Arabian Sea. As the North-East monsoon recedes, the area is exposed to tidal incursion of saline water from the Arabian Sea making the ecosystem predominantly saline. In order to prevent the saline incursion from the arabian sea, a salt water regulator (bund) was constructed in the lake which divides the Vembanadu estuary into a fresh water lake on the southern side and a saline lagoon in the north. Every year the shutter of the regulators is closed in December and opens in March and the impact of closure and opening of the regulator has been a topic of endless debate. When the regulator is closed there is virtually no flow of water beyond it on the southern side making this entire region as a static pool. The periodic tidal inflow, which used to flush the water body, is completely prevented with the result the drained water from the surrounding rice fields and human dwellings with heavy load of pollutants remains stagnant in the water body.

Studies were carried out from October 2004 to September 2005 in Kumarakom region of Vembanadu Lake. Monthly collections of estuarine water samples were made from 5 stations on the southern (inside the bund) and 5 on Northern (outside the bund) sides of the salt water regulator in sterile plastic bottles. Water samples were transported to the laboratory in an ice box and subjected to bacteriological examination within 2 hours of collection.

Bacteriological analysis

A three tube fermentation method was used to estimate faecal Coliform using ec broth (hi-media, Bombay, India) as the medium and incubation at 44.5°C for 24-48 hours. Loopful of culture from each tube showing growth and gas production were streaked on eosine methylene blue (emb, hi-media) agar for the isolation of E. coli and incubated at 37°C for 24 hours. Typical E. coli like cultures were isolated, restreaked to ensure purity and confirmed by indole, methyle red, voges proskauer and citrate (imvic) test. Isolates showing + + - - reaction for imvic test were confirmed as E. coli.

Two methods for enumeration of V. parahaemolyticus and V. cholerae were used. The first was a direct plating procedure, which included inoculating 0.2 ml estuarine water sample on thiosulfate citrate bile salts sucrose agar (tcbs, hi-media) agar plates, and incubating at 37°C for 48 hrs. Blue-green colonies were recorded as V. parahaemolyticus and yellow colonies were
considered as *V. cholerae* and held for further biochemical testing. In second method 10ml of estuarine water samples were inoculated into 40 ml alkaline peptone water for pre-enrichment in a conical flask and incubated at 37°C for 24 hours. Flasks showing growth in enrichment broths were streaked onto tbs agar and incubated at 37°C for 24-48 hours. Typical colonies, whenever present, were isolated, restreaked to ensure purity and maintained on nutrient agar slants for further biochemical characterisation.

The cultures were identified according to bacteriological analytical manual (bam) of United States food and drug administration (usfda). Cytochrome oxidase (+), nitrate reduction (+), Voges-proskauer (-) acid from sucrose (-) and lactose (-), growth in peptone water containing 0% (-), 3% (+), 6% (+) and 8% (+) NACL and growth at 43°C in lia (+) were considered as *V. parahaemolyticus*. For *V. cholerae*, cytochrome oxidase (+), nitrate reduction (+) voges-proskauer (+) acid from sucrose (+) and lactose (-), growth in peptone water containing 0% (+), 3% (+), 6% (-) and 8% (-) nacl and growth at 43°C in lia (+) were considered confirmatory.

For the detection of *salmonella*, about 1 or 2 litre volumes of surface waters were filtered through sterile 0.45-μm membrane filters. The filters were cut into pieces and placed into selective enrichment broth such as tetrahitonate broth (ttb, hi-media) and selenite cystine broth (scb, hi-media) following aseptic procedures and incubated at 37°C for 24-48 hrs. After selective enrichment, a loopful of cultures from both scb and ttb were streaked on to selective media such as xylose lysine deoxycholate (xld, hi-media) agar and hektoen enteric agar (hea, hi-media) plates and incubated at 37°C for 24 to 48 hours. Typical *Salmonella* like colonies were picked up restreaked to ensure purity and were maintained on tryptic soy agar (tsa, hi-media) slants at room temperature for further biochemical testing.

The stored *Salmonella* cultures were subjected to primary biochemical testing involving reactions in triple sugar iron (tsi, hi-media) agar, lysine iron agar (lia, hi-media) slants, indole production in Tryptone broth and urease production on Christnsen’s urea agar (hea, hi-media) were tested. Cultures matching typical reaction of *Salmonella* were subjected to secondary biochemical characterization involving fermentation of carbohydrates such as lactose, sucrose, Dulcitol and Salicin. Isolates that matched typical biochemical reactions of *Salmonella* were further confirmed by slide agglutination test using *Salmonella* polyvalent ’o’serum (DIFCO, USA). Serotyping of the strains was carried out at national *Salmonella* and *Escherichia* Centre, Kasauli, Himachal Pradesh, India.

**RESULTS AND DISCUSSION**

The present study has been taken up considering the modified flow patterns of the Vembanad lake due to the construction of a salt water regulator and public health importance as large number of people in this region use this water body for their day to day activities that include fishing for livelihood, transportation and recreation. The MPN index of faecal indicator bacteria such as faecal Coliform (Fc) at different stations during the period October 2004 to September 2005 is represented in figure 1. Levels of faecal Coliform contamination remained high throughout the study period and ranged from mean MPN value 1718-7706/100 ml and showed spatial variation at different stations along the southern and northern sides of the regulator. The MPN index of Fc was higher than those recorded in Cochin region (Lakshmanaperualsamy et al., 1981; Hatha et al., 2004) of Vembanad Lake and in Mondovi Zuary estuaries of Goa (Row 1981). The higher load of Fc in the study area is possible, as the human population has grown considerably in the last two decades and increased the waste generation. Many illegal sewers, especially from markets and septic tank effluent directly entering into the estuary. Also, there is a practice of dumping animal carcasses into the lake at various points near the salt water regulator. There are no effective measures to control such activities, which could result in gross contamination of the system, and the self purifying capacity of the system might cease to function once the waste load into this water body exceeds its carrying capacity.

Monsoon is a very prominent annual climatic feature of Kerala and hence the study period has been divided into three distinct seasons, such as pre-monsoon (February to May), monsoon (June to September) and post monsoon (October to January). Seasonal variation of indicator bacteria at each station during different seasons is represented in figure 2. The results indicate substantially high levels of indicator bacteria in southern part of the lake (inside region of salt water regulator) during monsoon than the northern region. Higher bacterial population during these months may be due to increased land run off during monsoon period resulting in a higher faecal input in to the lake from various sources. In our (Abhirosh and Hatha 2005) previous studies on the inactivation kinetics of indicator bacteria in Cochin Estuary we could find that sunlight was a major factor affecting the self-purifying capacity of the natural waters. Reduced intensity of sunlight due to overcast conditions during monsoon as well as increased turbidity from land run off resulting in reduced penetration of sunlight might the extended survival of faecal Indicator bacteria. An increase in the faecal Coliform level after rainfall was reported previously (Shehane et al., 2005).
Figure 1. MPN index of faecal indicator bacteria in northern and southern sides of salt water regulator in Vembanadu lake during 2004 to September 2005.

Figure 2. Seasonal variation of indicator Bacteria at different station in northern and southern side of salt water regulator in Vembanadu lake during Oct. 2004. Sept 2005.
However in the northern region (outside region of salt water regulator) more dynamic environmental condition prevailing (especially salinity) and the monsoon rains may dilute the bacterial load that enters into the lake. During December to march the system is closed and natural flow is prevented which results in the accumulation of organic load along the inner side of the bund, giving proper environmental conditions for the multiplication of bacteria. The use of this water for harvesting shellfish and recreation during this period may pose serious health risk to the people. After opening the salt water regulator in March, a sudden increase in the MPN index of Fc was observed at the stations outside the salt water regulator. During the summer months of March through May, tidal incursions to the stations outside the regulator, however, resulted in a decrease in fc values possibly due to the high salinity.

High densities of faecal indicator bacteria have been sporadically reported from different coastal regions in India (Pradeep and Lakshmanperumalsamy 1986). Other reports also indicated relatively low levels of total viable counts (TVC) and fc at several locations along the taminadu coast extending between the pulicat lake and the Cauvery river confluence (Venkateswaran and Natarajan 1987) in the port region of Bhavanagar (Abhay Kumar and Dube 1995) and coast of Visakapatnam (Clark et al., 2003). Faecal pollution of recreational waters may be a health hazard for bathers due to the presence of several microbial pathogens, including bacteria, viruses, fungi and protozoa.

Bacterial pathogens such as *V. cholerae*, *V. parahaemolyticus* and various serotypes of *salmonella* and indicator bacteria, *E. coli* were isolated and identified from various sampling stations. *Salmonella* serotypes included *Salmonella paratyphi* a, b, c and *S. Newport*. The percentage incidences of these specific pathogenic bacteria are represented in Table 1. No significant variation was observed in the prevalence level of specific pathogens at different stations along both sides of the salt water regulator, though there was slightly higher level of incidence of *Salmonella* at stations inside the regulator region. While *E. coli* were isolated consistently from all stations, the prevalence of pathogenic *Vibrios* varied from 32 to 45%. Prevalence of *Salmonella* was also relatively high (42-57%). The relatively high levels of prevalence of pathogenic bacteria in this important water body suggest high influx of sewage as well as good survival capabilities of these microorganisms to changing hydrographic parameters. The analysis of the results also indicated that there is no significant correlation between high levels of Fc and incidence of specific pathogens at various stations in the study area.

Table 1. Incidence of indicator and pathogenic bacteria in the Vembanadu lake during the study period

<table>
<thead>
<tr>
<th>Indicator/ Pathogenic bacteria</th>
<th>% incidence at stations inside the salt water regulator</th>
<th>% incidence at stations outside the salt water regulator</th>
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<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>86.7</td>
<td>85.6</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>57*</td>
<td>42**</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>32</td>
<td>31.5</td>
</tr>
</tbody>
</table>

*serotypes include *s. Paratyphi* a, s. Paratyphi b, s. Paratyphi c and *s. Newport*  
**serotypes include *s. Paratyphi* a, s. Paratyphi b and *s. Paratyphi* c

![Map showing Tannirmukkam salt water regulator and sampling locations](image)
The association of *V. parahaemolyticus* with freshwater fishes and their ability to survive in association with freshwater plankton under certain environmental conditions is significant. Recent studies on the Na⁺ requirement of *V. parahaemolyticus* and *V. cholerae* have indicated that, in contrast with other marine bacteria, the quantitative requirements for Na⁺ for growth vary with the substrate serving as the carbon and energy source in the medium (R. A. Macleod, personal communication). This would imply that, under certain specific nutrient conditions, the Na⁺ requirement of *V. parahaemolyticus* is not mandatory and that the halophile can well survive in conditions where the salt concentration may be equal to or even lower than physiological concentrations. Several workers reported the high incidence of *Vibrio* Sp. From Indian sub continent. For instance, high densities of *Vibrio* Sp. were reported from the inshore waters of the East Coast (Nair et al., 1980) from Cochin back waters (Chandrika 1983) and from the offshore waters of the West Coast (Pradeep and Lakshmanperumasamy, 1986; Lokabharathi et al., 1987) and from Visakaputtanam (Clark et al., 2003). However, the prevalence levels were lower when compared to our findings.

In the present study, the prevalence of *Salmonella* was significantly higher than those reported by (Hatha et al., 2004) from the Cochin estuary which is northern part of the Vembanadu lake system. However, diversity of Serovars was limited. While *S. newport* was the predominant Serovar, others included *S. paratyphi* a, b and c. Though *salmonella* cells may enter into viable, but non-culturale state (VBNC) under conditions of stress, their presence would be a health concern especially in shell fish growing waters. The study area is well known for its shellfish resources and many people are engaged in exploiting this important fishery resource for their livelihood. While the flesh is being sold for human consumption in the local markets, the shell is treated to produce powdered calcium carbonate. In many cases the infection due to *Salmonella* are linked to the consumption of seafood (centers for disease control and prevention, 2000) particularly shellfish (Heinitz et al., 2000). Though food and water borne outbreaks are common in the study area, no systematic reporting, tracking and monitoring mechanisms are in place in the study area. The *Salmonella* enterica serovars isolated in the present study such as *S. paratyphi* a, b, c and *S. Newport* are important human enteric pathogens causing enteric fever by the contamination of water and food. Since humans are the only reservoir of *S. paratyphi* a (hook 1990) the results are indicative of the contamination of this water body from human excreta. This is the first report of the isolation of these pathogenic strains of *Salmonella* from the Kumarakom region of Vembandu lake.

The consistently high load of *E. coli* and its isolation from all the stations indicate that the water body is undergoing severe sewage pollution. Although we do not have the information about the serotypes of *E. coli* from the present study, we have encountered more than 40 serotypes of *E. coli* including potential pathogens such as enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and Uropathogenic *E. coli* (UPEC) in our previous studies at Cochin estuary (Hatha et al., 2004). We also expect such a wide diversity of *E. coli* serotypes including Diarrhogenic strains in the present study area.

REFERENCES


