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IN SITU SURVIVAL OF INDICATOR BACTERIA AND ENTERIC PATHOGENS IN A TROPICAL ESTUARY

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ABSTRACT

In the present investigation experiments have been conducted to evaluate the survival of *E. coli*, *S. paratyphi* and *V. parahaemolyticus* as a function of biotic and chemical factors in estuarine water under *in situ* condition using membrane diffusion chamber. Survival capacity of *S. paratyphi* and *V. parahaemolyticus* in relation to *E. coli* has been worked out in order to see whether *E. coli* can be considered as valid indicator for *Salmonella* and *V. parahaemolyticus* in estuarine waters. *In situ* experiments using membrane diffusion chamber revealed the effect of biological factors especially protozoan and other predators on the removal of the test organisms in aquatic environments. The results also showed that the chemical composition of the estuarine water was suitable for their survival. The comparative survival experiment revealed that the survival of *E. coli* was low compared to *V. parahaemolyticus* and *S. paratyphi* in water. Hence the use of *E. coli* as an indicator for the presence of pathogenic bacteria such as *V. parahaemolyticus* and *Salmonella* in aquatic environments needs to be reconsidered at least in the present study area.

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INTRODUCTION

Rapid urban and industrial growth has resulted in a vast quantity of potentially harmful waste being released into the environment. Large number of faecal microorganisms, including some pathogenic bacteria, protozoa and viruses are discharged into natural waters through sewage outfall and other types of faecal discharges. Pathogenic organisms found in sewage can adversely affect public health when humans come in contact with water while wading, swimming, fishing, drinking etc.

The behavior of enteric microorganisms in the coastal environment depends on many factors that have been investigated by several workers. These factors include water temperature (Anderson *et al*., 1983), adsorption and sedimentation processes (Auer and Niehaus, 1993), sunlight action (Sinton *et al*., 1999), lack of nutrients, predation by bacteria and protozoa (Hahn and Hofle, 2001), bacteriophage lysis (Ricca and Cooney, 1999), competition with autochthonous microbiota and antibiosis.

Since it is difficult to imitate these various dynamic environmental factors in the laboratory conditions the survival and fate of indicator and enteric pathogens in aquatic environments were studied *in situ* using membrane diffusion chamber. The specific objective is to study the *in situ* survival of *E. coli, S. paratyphi* and *V. parahaemolyticus* in estuarine water using membrane diffusion chamber.

MATERIALS AND METHODS

E. coli, *S. paratyphi* and *V. parahaemolyticus* isolated from the Kumarakom estuary were used for this study. Inocula of the test microorganisms were prepared by growing *E. coli*, *S. paratyphi* and *V. parahaemolyticus* separately in 10mL sterile Tryptone Soya Broth (TSB) for 24hr at 37°C. After incubation the cells were harvested by centrifugation at 3000 rpm for 15 minutes and washed twice with sterile isotonic saline. After the final wash the cells were resuspended in 10mL sterile isotonic saline at a concentration of 10^8 colony-forming units per mL. From this final suspension 1mL was inoculated into 250 mL erlenmeyer flask with 100mL of the test solution. Percentage survivors and injured cells were assayed after 1, 2 and 3 days using spread plate technique.

The membrane diffusion chambers were constructed of plexiglass of 12mm thickness. The plexiglass parts of the chambers were sterilized by autoclaving, and the membranes (0.4μm) were irradiated with ultraviolet light. In this chamber, porous membranes retain a viable suspension of bacteria in a natural or artificial aqueous environment for study or enumeration. The membrane allows the water and solutes in that environment to diffuse readily through the chamber and to interact with the bacterial suspension. A significant advantage of this system over procedures in which bacteria are studied in a limited and unchanging water sample is that a continuous exchange of water and solutes come in contact with the bacteria under investigation.

Individual membrane diffusion chambers loaded separately with 1 mL of washed cells of *E. coli*, *S. paratyphi*, *V. parahaemolyticus* were placed in lake, at sites near the vicinity of the Thanneermukkam barrage. To study the effect of biological factors on the test organisms, raw estuarine water collected from the lake were introduced into the chamber along with washed test organisms. In another chamber test organisms suspended in sterile estuarine water was used as a control. Heavy string was used to suspend the chambers from an overhead support. This allowed some rotational movement of the chambers within the lake. One mL of sample was removed daily in between 7 -9 am with sterile syringe for bacterial enumeration for a period of three days. At the same time, the water samples were collected from the lake to study the physiochemical properties of the lake water. The physiochemical characteristics such as temperature, pH, conductivity, acidity, alkalinity, hardness, chloride and salinity were studied as per APHA (1998).

RESULTS AND DISCUSSION

The study area, Kumarakom estuary, had a mean salinity of 10.1 ppt and dissolved oxygen (DO) content 6.7 mg/L. The mean values of physiochemical parameters of estuarine water during the study periods is given

in Table 1. Die-off of enteric bacteria in estuarine environments has been broadly attributed to a variety of interacting physical, chemical and biological factors and processes. Although understanding these factors and their relative importance is justifiable purely on an ecological basis, in practical terms such studies are necessary to assess the validity and suitability of the fecal coliform group or other microbial indicators which may be used as quantitative measures of fecal pollution in shellfish-growing waters.

Percentage of survival and injury of *E. coli, S. paratyphi* and *V. parahaemolyticus* under *in situ* condition is presented in Table 2. The percentage survival was found to be decreasing as the time progressed. *V. parahaemolyticus* showed better survival followed by *E. coli* and *S. paratyphi*. However, the percentage of injury showed temporal variations for each organism. For *E. coli* the level of injury was constant during the second and third day while *S. paratyphi* exhibited relatively lower injury at the beginning which was found

Table 1: The mean values of physiochemical parameters of estuarine water during the study period

Parameters	Values (Mean)
Water temperature (°C)	29°C
pH	6.5
Conductivity	1.73 mS
Acidity	30 mg $CaCO_3$/l
Alkalinity	19 mg $CaCO_3$/l
Hardness	7600 mg/l
Salinity	10.1 ppt
Dissolved oxygen	6.7 mgO_2/l

Table 2: Percentage of survival and injury of *E. coli, S. paratyphi* and *V. parahaemolyticus* in raw estuarine water under field conditions

Test micro-organisms	Percentage of survival days			Percentage of injury days		
	1	2	3	1	2	3
E. coli	6.83	3.1	1.1	60	69	69
S. paratyphi	9.5	5.1	0.8	49	57	79
V. parahaemolyticus	20.2	8	5.8	68	58	62

to be increasing as the time progressed. But in the case of *V. parahaemolyticus* the fraction of injured cells were lower on 2nd day when compared to 3rd day.

Relative survival curves of *E. coli*, *V. parahaemolyticus* and *S. paratyphi* in raw and sterile estuarine water under *in situ* condition is shown in Fig. 1 and 2. The results indicated that the cells suspended in raw estuarine water declined rapidly when compared to cells suspended in sterile estuarine water. The experiment started with around 10^8 cells of the test organisms, which reduced almost 2 logs in 3 days in raw estuarine water and almost 1 log in sterile estuarine water. Among the bacteria *S. paratyphi* showed slightly enhanced

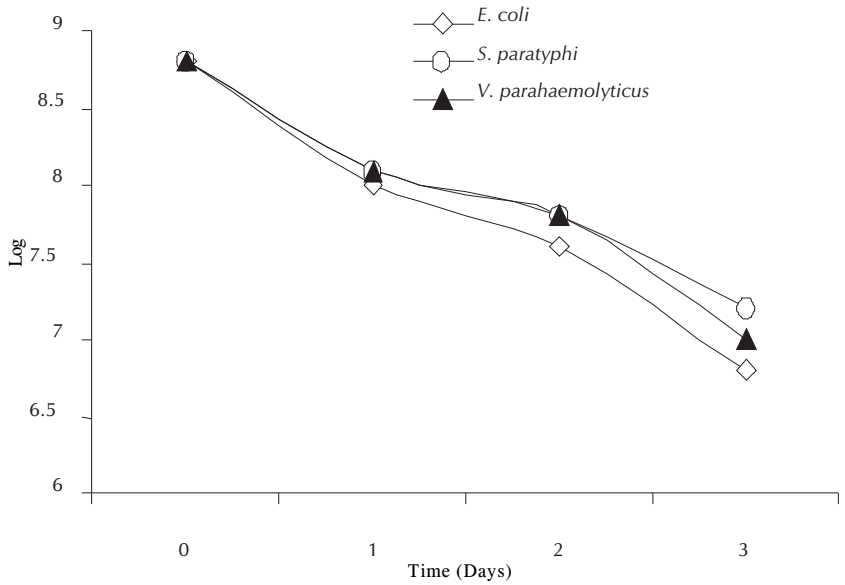

Figure 1: *In situ* survival curves of *E. coli, S. paratyphi* and *V. parahaemolyticus* in raw estuarine water

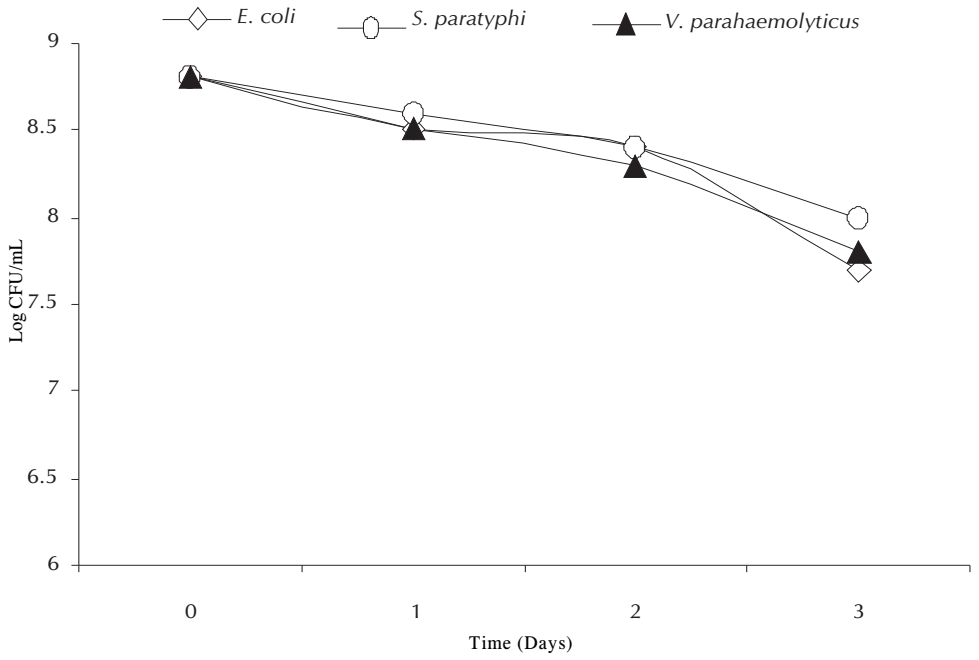

Figure 2: In situ survival curves of E. coli, S. paratyphi and V. parahaemolyticus in sterile estuarine water

survival capacity followed by *V. parahaemolyticus* and *E. coli* in raw estuarine water and in sterile estuarine water. There was no significant variation on the survival of test organisms in raw estuarine water.

The percentage survival was found to be decreasing as the time progressed. *V. parahaemolyticus* showed better survival followed by *E. coli* and *S. paratyphi*. However, the percentage of injury showed temporal variations for each organism. For *E. coli* the level of injury was constant during the second and third day while *S. paratyphi* exhibited relatively lower injury at the beginning which was found to be increasing as the time progressed. But in the case of *V. parahaemolyticus* the fractions of injured cells were lower on 2nd day when compared to 3rd day.

In membrane diffusion chamber the bacteria under investigation come in contact with a continuous exchange of water and solutes and the membrane prevents the entry of native bacteria into the diffusion chamber. Test microorganisms inoculated into raw estuarine water with all of its biological factors showed a rapid reduction in the number of cells, while they showed an enhanced survival in sterile estuarine water. This indicates that the protozoan and other autochthonous microorganisms present in the raw estuarine water cause the inactivation of test microorganisms. The enhanced survival in sterile water also revealed that the chemical composition of the stuarine water was found to be suitable for the survival of the above microorganisms in estuarine water. Important physical factors such as dilution and mixing of the estuarine water were also allowed to operate by keeping the test microorganisms in the membrane diffusion chamber, which permitted the movement of water.

In situ experiments using membrane diffusion chamber revealed the effect of biological factors especially protozoan and other predators on the removal of the test organisms in aquatic environments. The results also showed that the chemical composition of the estuarine water was suitable for their survival. The comparative survival experiment revealed that the survival of *E. coli* was low compared to *S. paratyphi* and *V. parahaemolyticus* in water. Hence the use of *E. coli* as an indicator for the presence of pathogenic bacteria such as *V. parahaemolyticus* and *Salmonella* in aquatic environments needs to be reconsidered at least in the present study area.

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