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Occurrence and distribution of *Clostridium perferingens* in relation to conventional faecal indicator bacterial in Shrimp farm

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Biomonitors indicate the microbiological quality of water. Though *E. coli* is a microbiological indicator, it cannot be considered as a perfect one. In this regard, attempts for finding a new indicator of faecal pollution in place of *E. coli* was carried out by performing a comparative study between *E. coli* and *Clostridium perfringens* as faecal indicator in shrimp farms. The presence of these organisms was determined by evaluating the brackish water and shrimp samples from various areas of Cochin, using MPN technique. The effect of disinfectant chlorine was examined over these organisms which revealed the sensitive nature of *E. coli*. *Clostridium perfringens* was found to be highly resistant in comparison with *E. coli* and thus suggesting it as a better indicator of faecal contamination in brackish water to evaluate the microbial quality of shrimp farms than *E. coli*.

Key words : Biomonitor, *E. coli*, *Clostridium perfringens*, Brackish water, Shrimp, MPN technique, Chlorine.

INTRODUCTION

Aquaculture has recently emerged as an alternative to the decline in marine resources. Shrimp, being the most widely cultured variety in India, plays an important role in the growth of seafood exports. The single most significant factor causing major decline in the population of many fish species is pollution.

Pathogens are always present in the aquatic environment. The main concern therefore, has been in introducing new disease organisms or causing environment deterioration that can result in increased population of indigenous pathogens (Reid *et al.*, 1976).

Biomonitors are used to indicate the microbiological quality of water. Traditionally *E. coli* has been the chosen indicator of faecal pollution due to its occurrence in large numbers than other organisms. But *Clostridium perfringens* was found to persist for relatively longer time in the environment compared to coliforms (Herbett, 1992). As *E. coli* cannot be considered a perfect indicator, an investigatory attempt was done for searching a new indicator of faecal contamination to substitute *E. coli* by comparing *E. coli* and *Clostridium perfringens* as faecal indicators in shrimp farms.

MATERIALS AND METHODS

Sample preparation and processing:

Water and shrimp samples were collected from two brackish water farms, namely Chellanum and Vaippin in and around the areas of Cochin. Shrimp sample collected include *Penaeus monodo*n and *Penaueus indicus*. The sample preparation involved bringing shrimps in a sterile polythene bag and further the whole part being into small pieces under aseptic condition using scalpel.10gm of sample was then homogenated in 100 ml of sterile physiological saline (0.85% NaCl). For processing the sample, 5 tube and 3 tube (MPN) method was followed to enumerate the amount of *E. coli* and *Clostridium perfringens* in the given sample.

Coliform isolation:

Isolation of *E. coli* was carried out using the presumptive, confirmatory and completed test 3 tube and 5 tube method dilution were done for fish and water sample by inoculating MacConkey broth, with 10ml, 1ml, 0.1ml aliquots of homogenates and 10ml, 1ml, 0.1ml, 0.01ml of water sample, respectively and incubating the tubes at 37^0C for 24 hours. Positive tubes were confirmed by inoculating into Brilliant green lactose bile broth and incubating at 37^0C for 24 hours. The completed test involved transferring a loop full of inoculum from the positive tubes to E.C and tryptone broth and incubating at 46^0C for 24 hours.

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Isolation of Clostridium perfringens:
A similar procedure of presumptive, confirmatory and completed test was carried out for the isolation. Presumptive test was done by inoculating 10ml, 1ml, 0.1ml aliquots of homogenates into lactose sulphite broth and incubating under anaerobic conditions at 46^{0}C for 24 hours. For confirmation inoculation was carried out into iron agar milk medium from the positive tubes, followed by incubation under anaerobic condition at 46^{0}C for 24 hours. The completed test involved, streaking cultures from the positive tubes on tryptose sulphite cycloserine agar and five colonies of *Clostridium perfringens* were further characterized by testing for carbohydrate fermentation, reduction of motility nitrate and gelatin liquefaction.

Determination of Clostridium perfringens type A toxin:
Tryptose sulphite cycloserine agar plate with egg yolk was used for the determination. Antitoxin as spread on one part and marked as A, the other part without antitoxin marked as B. Single line streak was made from the part without antitoxin to the part containing antitoxin by taking a loop full of culture from thioglycollate broth. Anaerobic incubation of plates was accomplished in spray's dishes were using alkaline pyrogallol method (Spray, 1930) and spray's dishes placed at 37^{0}C for 24 hours.

Chlorination procedure:
500ml of water was taken, (10ppm) of chlorine was prepared and 7ml pipetted into the tub containing 15 to 20 prawn samples. Upon 15 minutes, 0.025% sodium thioglycollate was added to neutralize the effect of chlorine and further kept for 5 minutes. The samples were then taken and processed for the enumeration of *Clostridium perfringens* and *E. coli*.

Estimation of salinity:
1-2 drops of potassium chromate indicator was added into a flask containing 5ml sample and few ml distilled water. Silver nitrate was then titrated against the taken sample. The appearance of yellow to pale pink color was taken as the end point and the final burette reading was noted.

RESULTS AND DISCUSSION

Bacteria other than Coliform, *Escherichia coli* group are often considered as agents to monitor faecal pollution. Faecal *Streptococci* and *Clostridium perfringens* have also been estimated in brackish and fresh water farms (Lalitha *et al*., 1986). In the present study, enumeration of *E. coli* and *Clostridium perfringens* was done by 5 tube / 3 tube MPN method to estimate the amount of these organisms in the brackish water farm to monitor faecal pollution. Faecal coliform level associated with sewage discharge may be reduced by sewage treatment process, chlorination and other disinfectant practices. There is no assurance that coliform free water is free of microbial pathogens.

Previous investigation has reported that total coliform level in water and prawn sample in brackish water was 40, 140, respectively per 100 ml (Surendran *et al.*, 2000). In the present investigation, the *E. coli* count were 1800 +/ 100ml, 1600 +/ 100ml, 1800 +/100ml, 30+/100ml, in the water sample and 25+/g, 140+/g, 140+/g, 25+/g, in fish sample (Table 1). The high level of *E. coli* is due to faecal

Table 1 : The amount of *E.coli* in water and fish sample by 5 tube / 3 tube method

Samples	*E.coli* count	
	Water (/100 ml)	Fish sample (/ g)
1.	1800	25
2.	1600	140
3.	1800	140
4.	30	25

Table 2 : The amount of *Clostridium perfringens* in water and fish samples by 5 tube / 3 tube method

Samples	*Clostridium perfringens* count	
	Water (/100 ml)	Fish sample (/ g)
1.	0	2.5
2.	12	0
3.	0	0
4.	0	2.5

contamination in the farm. The *Clostridium perfringens* was only in small amount in first fish sample and fourth fish sample and the amount is lower in water sample (Table 2).

Camper *et al.* (1979) had suggested in his previous studies that a concentration of 0.5mg of chlorine per liter gave reproducible and predictable chlorine injury in *E. coli*. 90% injury rate was obtained in 8 minutes of chlorine exposure. Also the resistance of Clostridial spores to 0.5ppm of available chlorine at 10^{0}C suggested by Dye and Mead (1972) was; *Clostridium perfringens* -

Log10 of mean viable count at time/hour

0,	½,	1,	2
8.4,	8.4,	8.4,	8.2

Similar data was obtained in the present study. In the chlorinated fish samples first and second there was a reduction of 87% and 90% *E. coli* (Table 3), than non-

Table 3 : The effect of chlorine on *E coli.*

Samples	*E.coli* count in CFU/ml	
	Before chlorine treatment	After chlorine treatment
1.	$140x10^5$	$9x10^5$
2.	$110x10^5$	$9.5x10^5$

Note: 10 ppm of chlorine prepared

Table 4 : The effect of chlorine on *Clostridium perfringens*

Samples	*Clostridium perfringens* count in CFU/ml	
	Before chlorine treatment	After chlorine treatment
1.	$2.5x\ 10^5$	$2.5x\ 10^5$
2.	$2.5x\ 10^5$	$2.5x\ 10^5$

chlorinated samples, but no reduction was noticed in the amount of *Clostridium perfringens* before and after chlorination (Table 4).

The feasibility of semi-intensive culture of shrimp in low saline environment was investigated by Shyamalendu *et al*. (1999) and the yield of shrimp was found high in low saline water (15-28ppt).In the present study the salinity was checked for water samples 1 and 2, and it was 16.58 and 16.20 ppt (Table 4). This indicates that brackish water is suitable for prawn culture. *Clostridium perfringens* type A is a potent food poisoning organism and is ubiquitous. Standard biochemical tests were used to confirm *Clostridium perfringens* type A.

In conclusion it may be stated that, water bodies of the inland areas pose an alarming scenario, so far as faecal pollution is concerned. The pollution rate is higher in brackish water. To overcome this situation there should be a regular monitoring of aquatic system for pollutants and pathogens. The present study proved that continuous use of disinfectant lowers the amount of *E. coli*, but there was no reduction in the amount of Clostridium perfringens. In this sense *Clostridium perfringens* can be used as better indicator of faecal contamination than *E. coli* in brackish water and also in food processing industry.

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