Distribution of *Clostridium perfringens* in Fish and Aquatic Environments

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Clostridium perfringens has been implicated as the etiological agent in many food poisoning outbreaks. It is a Gram-positive anaerobic spore-forming bacillus. Spores of *C. perfringens* are widely distributed in soil, sewage, aquatic environments, animal and human intestine, and their presence is usually indicative of sewage contamination. The distribution of *C. perfringens* in water, sediment and fish/shellfish from brackish water and freshwater environments was investigated. Water, sediment and fish/shellfish samples collected from various locations in three districts of Kerala were screened for the presence of *C. perfringens*. The study revealed that incidence of *C. perfringens* was high in sediment samples compared to water samples. In farm sediment samples, occurrence of *C. perfringens* was high (30%), and 22% of the fish/shellfish samples also harboured *C. perfringens*.

Key words : Fish, aquatic environment, *and Clostridium perfringens*

Clostridium perfringens is an anaerobic enteric pathogen, widely distributed in soil, water, sediments, intestines of animals and humans world over (Matches *et al.*, 1974; Cabelli, 1978; Labbe, 1991; Songer, 1996; Bothner, 2000). This organism is of concern to the food industry because of its involvement in several human food-borne illnesses. In terms of number of cases of bacterial food borne illness reported each year, *C. perfringens* generally ranks second in Canada and the United Kingdom and third in the United States (Labbe, 1989; Huss, 1994; Mc Clane, 2001). In most cases, the foods involved are cooked meat or poultry products containing high counts of viable cells (Labbe & Harmon, 1992). Fish are not commonly involved in food poisoning outbreaks caused by this organism. However, both raw and processed seafoods were implicated in *C. perfringens* food poisoning outbreaks in the United States and Japan (Taniguti, 1971; Bryan, 1980; Caico, 1998).

Contamination of fish/shellfish by *C. perfringens* has been reported by Taniguti (1971; 1978), Madden, *et al.* (1986), Easterbrook & West (1987), Saito (1990), Lalitha *et al.* (1990), Burkhardt & Calci (2000). Consumption

of raw or partially cooked shellfish resulted in *C. perfringens* food poisoning in the United States and Japan (Caico, 1998). Information on the occurrence of this pathogen/indicator organism in brackish water and freshwater environments in India is scanty. The aim of the present study was to investigate the incidence and levels of *C. perfringens* in fish/shellfish from brackish water and freshwater environments in order to identify the source of contamination and to monitor microbial risks associated with consumption of fish/shellfish.

Materials and Methods

The study covered 3 major districts of Kerala. Samples were collected from Perumpadappu, Chellanam, Vypeen and Udayamperoor in Ernakulam district, Vallakam in Kottayam district and Ashtamichira in Trichur district of Kerala. The study was carried out over a period of 3 years from 1998 to 2001. Water, sediment and fish/shellfish were collected from various locations as described elsewhere (Surendran *et al.*, 2000) and screened for the presence of *C. perfringens* by the Most Probable Number (MPN) method using lactose sulphite (LS) broth (West, 1989). Presence of *C. perfringens* was confirmed by streaking cultures from positive MPN tubes on tryptose sulphite cycloserine (TSC) agar and further characterization by biochemical tests as per USFDA (Rhodehamel & Harmon, 1998).

Results and Discussion

Table 1 shows *C. perfringens* counts in water and sediment collected from brackish water and freshwater environments. *C. perfringens* numbers (MPN) ranged from 0-90.100ml^{-1} of water (mean: 5). Of the 33 water samples collected from aquaculture farms (Table 2), the densities of *C. perfringens* were 0-110.100ml^{-1} (mean: 5). In sediment samples, *C. perfringens* counts ranged from 0-110000.100g^{-1} (mean: <7000.100g^{-1}) and incidence was 27%. The counts were always higher than that of the water from the corresponding area. In farm sediments (Table 2), *C. perfringens* numbers ranged from 0-600000.100g^{-1} (mean: 15800.100g^{-1}). The relative frequencies and counts of *C. perfringens* in fish/shellfish studied are given in Table 3. Contamination by *C. perfringens* was present in 22% of the 48 samples of fish/shellfish analysed. Incidence in farm samples was 19%. In fish, *C. perfringens* counts ranged from 0-6000.100g^{-1} (mean: 860.100g^{-1}). In wild shellfish, *C. perfringens* numbers were in the range of 0-4500.100g^{-1} (mean: 1100.100g^{-1}) whereas in farmed shrimp, counts ranged from 0 to 14000.100g^{-1} (mean: 1800.100g^{-1}).

Table 1. Most Probable Number of *Clostridium perfringens* in brackish water and freshwater environments

		Sampling sites					
		Perumpadappu	Vypeen	Chellanam	Udayamperoor	Vallakam	Ashtamichira
Water							
No. of samples		4	7	5	3	5	1
MPN.100ml^{-1}	mean	22	1	5	-	5	-
	range	0-90	0-10	0-25	-	0-25	-
Sediment							
No. of samples		-	4	5	3	5	1
MPN.100g^{-1}	mean	-	11200	22000	1500	3700	-
	range	-	0-45000	0-110000	0-4500	0-14000	-

The mean counts of *C. perfringens* recorded for inland water samples in the present study is 5.100ml^{-1}. Similar results were reported by Payment & Franco (1993) and Cabelli (1978). Similar studies conducted by Bison & Cabelli (1979) in the aquatic environments of Rhode Island, USA and by Payment & Franco (1993) in the river water in Canada reported comparatively higher values (1000-126900 cfu.100ml^{-1}) for *C. perfringens*. Contamination of river and near shore waters was frequent (93-100%) in Japan (Taniguti, 1978; Saito, 1990) but only 40% of river mouth water samples harboured enterotoxigenic strains (Saito, 1990). The mean densities of *C. perfringens* in the Gulf Coast (USA) estuarine water, where shellfish were grown, were 19±2.8. 100ml^{-1} (Burkhardt & Calci, 2000). Unlike faecal coliforms, there are no established standards for using *C. perfringens* densities to assess the sanitary quality of shellfish or shellfish growing waters. This is attributed

Table 2. Most Probable Number of *Clostridium perfringens* in brackishwater and freshwater farms

		Sampling sites				
		Vypeen	Chellanam	Udayamperoor	Vallakam	Ashtamichira
Water						
No. of samples		11	12	4	5	1
MPN.100ml^{-1}	mean	11	4	3	0.4	-
	range	0-110	0-45	0-11	0-2	-
Sediment						
No. of samples						
MPN.100g^{-1}	mean	58400	12400	5000	3100	-
	range	0-600000	0-110000	0-20000	0-11000	-

to the inability to distinguish the age of the contamination due to extreme stability of the organism in the environment (Burkhardt & Calci, 2000).

Table 3. Distribution of *Clostridium perfringens* in fish/shellfish from brackish water and freshwater environments of Kerala

Species	Sampling site	No. of samples tested	Percentage positive	*C. perfringens* MPN.100g^{-1} mean (range)
Wild caught fish/shellfish				
Villorita cyprinoides	Perumpadappu	3	33	1500 (0-4500)
Villorita cyprinoides	Vypeen	3	33	1500 (0-4500)
Villorita cyprinoides	Udayamperoor	4	25	400 (0-1500)
Oreochromis mossambicus	Vypeen	2	50	Sm: 1000 (0-2000)
Farmed fish/shellfish				
Penaeus monodon	Vypeen	5	40	Sm: 2500 (0-11000) Int: 20000 (0-90000)
Penaeus indicus	Vypeen	6	17	Sm: 400 (0-2500) Int: 18300 (0-110000)
Mugil cephalus	Vypeen	5	20	Sm: 1200 (0-6000) Int: 2800 (0-14000)
Penaeus monodon	Chellanam	12	8	90 (0-1100)
Oreochromis mossambicus	Udayamperoor	4	25	Sm: 400 (0-1500) Int: 7500 (0-30000)
Macrobrachium rosenbergii	Vallakam	3	33	Sm: 4600 (0-14000) Int: 36600 (0-110000)
Macrobrachium rosenbergii	Ashtamichira	1	ND	ND

Sm: skin/shell with muscle; Int: intestine, ND: not detected

Spores of *C. perfringens* serve as excellent tracers of sewage input into an ecosystem and higher *C. perfringens* populations (1000-150000.g^{-1}) found in surface sediments in Long Island Sound indicated widespread and long term addition of human waste to the sedimentary system (Mecray, 1999). The mean counts of *C. perfringens* were higher in farm sediments (mean: 15800.100g^{-1}) compared to brackish water sediments (7000.100g^{-1}). The feeder canals of fish farms can deposit these bacteria into the farm environment and vice versa. Previous studies on microbial pollution rate of Inland water bodies have shown an alarming rate of faecal pollution (Surendran *et al.*, 2000). They found higher load of coliforms and faecal coliforms in the sediments compared to the overlying water. It has been reported that *C. perfringens* spores persist in sediments over a long period of time (Hill *et al.*, 1993) which could perhaps explain the high numbers detected in the present study.

The relative frequency of *C. perfringens* in brackishwater and freshwater fish/shellfish samples analyzed were low (0-40%) in the present study compared to that reported earlier for seafood (68-88%) in Japan (Taniguti, 1971) and in India (Lalitha *et al.*, 1990). *C. perfringens* was detected in 2-3% of the seafood samples from San Francisco and Seattle retail markets in USA and the counts ranged from 5-60.g^{-1} (mean: 18.g^{-1}) (Foster *et al.*, 1977; Abeyta, 1983). The densities of *C. perfringens* in farm sediments and in the intestine portions of farmed shrimp were in the same range. Because farm sediments and shrimp faeces harbour *C. perfringens,* shrimp meat might get contaminated during processing. *Clostridium* spp. tolerate a wide range of temperatures and they were consistently isolated from intestine of farm-raised channel catfish (MacMillan & Santucci, 1990).

Bivalve molluscs may accumulate the pathogens to levels considerably greater than those in the overlying water (Metcalf *et al.*, 1979; Rippey *et al,* 1987; Burkhardt & Calci, 2000). In the present study, clams (*Villorita cyprinoides)* showed *C. perfringens* counts (0-4500.100g^{-1}; mean: 1100.100g^{-1}) greater than those in the growing waters (0-90.100ml^{-1}, mean: 5.100ml^{-1}). In bivalves, counts of *C. perfringens* spores were in the range of 10^2-10^4.100g^{-1} (Madden *et al.*, 1986; Easterbrook & West, 1987). The health hazards associated with the presence of low numbers of *C. perfringens* are probably quite small (Cabelli, 1978; Notermans *et al.*, 1995). The keys to minimise health risks associated with the presence of *C. perfringens* are prompt and proper refrigeration during storage and distribution, strict adherence to good manufacturing practices and sanitation, and reheating of leftovers of sensitive foods before consumption.

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