Distribution of *Clostridium perfringens* in Fishes and Prawns

LALITHA, K. V., UNNITHAN, G. R. and K. M. IYER
Central Institute of Fisheries Technology, Cochin - 682 029

Examination of skin and shell of raw fishes and prawns, collected from local landing centres and markets, revealed the occurrence of *Clostridium perfringens* on 47 of of the 66 samples. Only 27 samples showed presence of *C. perfringens* in the guts. Incidence of *C. perfringens* was high in prawn guts. *C. perfringens* formed less than one percent of the total clostridial population on fishes and prawns.

Clostridium perfringens is receiving greater attention in recent times in view of its close relationship with many food poisoning episodes. Published reports of outbreaks of *C. perfringens* food poisoning have come from United States, United Kingdom, Japan, Germany, New Zealand, Denmark and India (Hobbs, 1979). Bryan (1973) has reported that both raw and cooked fish are sometimes contaminated with *C. perfringens*. In Japan, fish dishes made from heated and processed fish products had contributed to *C. perfringens* food poisoning (Taniguti, 1971). *C. perfringens* food poisoning has occurred from imperfectly thawed frozen salmon steamed or boiled, left overnight in the liquor and eaten cold (Hobbs & Roberts, 1987).

Detection of *C. perfringens* in fishes has been made by Nichida *et al.* (1969), Taniguti (1971), Sohn *et al.* (1973), Matches *et al.* (1974), Burow (1974) and Bonde (1967). In India, attempts were made to study the incidence of *C. perfringens* in prawn canneries (Nambiar & Iyer, 1973) and fishes (Lalitha & Iyer, 1986).

The present paper is a report on the distribution of *C. perfringens* in raw fishes.

Materials and Methods

Fish samples procured from the local landing centres and also from the markets, in and around Cochin, were immediately brought to the laboratory in sterile polythene bags and analysed within 2–4 hours.

Most Probable Number method (3 tubes/dilution) using lactose sulphite (LS) broth (Beerens *et al.*, 1982) was followed for the enumeration of *C. perfringens*. Muscle with skin and intestine of fish samples were analysed separately. Sampling procedure, media and conditions used were the same as that of Lalitha & Iyer (1986).

Cultures from LS medium tubes showing positive growth were streaked on sulfite-cycloserine agar (Hauschild *et al.*, 1977) and five colonies of presumptive *C. perfringens* were selected after an anaerobic incubation of 24 h at 37°C. Selected colonies were further characterised by testing for fermentation of lactose, sucrose, glucose, raffinose and salicin, indole reaction, stormy clot reaction in litmus milk, reduction of nitrate, catalase production, motility and gelatin liquefaction (FDA, 1978). A control strain of *C. perfringens* (NCIB 6125) was used for interpretation of test reactions.

Table 1. *Distribution of total sulphite reducing clostridia and Clostridium perfringens in fishes and prawns (count per 10 g)*

Fish species	No. of samples	Skin/shell		Gut	
		Total clostridial count x 10^4	Count of *C. perfringens*	Total clostridial count x 10^4	Count of *C. perfrigens*
Lactarius lactarius	3	4.20*	7	80.0	6
Sardinella longiceps	10	3.90	8	8.0	520
Rastrelliger kanagurta	8	0.48	nd	8.3	nd
Johnius dussumieri	5	6.40	160	860.0	6,300
Nemipterus japonicus	6	6.50	12	970.0	nd
Tilapia mossambica	2	191.00	580	2,400.0	6,950
Mugil cephalus	4	5.90	5	52.0	nd
Saurida tumbil	1	0.28	110	690.0	11,100
Sillago sihama	2	11.00	120	250.0	46
Decapterus russelli	2	5.40	5	790.0	nd
Mugil parsia	2	6.30	1	10.0	2,300
Ottolithus argenteus	3	15.00	1	15.0	nd
Anodontostoma chacunda	2	6.00	nd	15.0	nd
Sardinella gibbosa	1	68.00	nd	110.0	nd
Arius sp.	1	0.20	nd	1.1	nd
Penaeus indicus	8	65.00	260	2,100.0	820
Metapenaeus dobsoni	6	43.00	280	1,220.0	5,440

* average values; nd = not detected

Table 2. *Frequency of incidence of C. perfringens on the surface of skin/shell of fishes and prawns (count/10g)*

Count of *C. perfringens*	No. of samples having total clostridial count of			
	10^3	10^4	10^5	10^6
Nil	5	6	7	1
1–10	3	9	12	—
11–100	—	3	4	2
101–500	1	1	7	—
501 and above	1	1	2	1

Table 3. *Frequency of incidence of C. perfringens count in the gut of fishes and prawns (count/10 g)*

Count of *C. perfringens*	No. of samples having total clostridial count of			
	10^4	10^5	10^6	10^7
Nil	10	13	12	4
10–100	4	—	3	2
101–1000	—	—	2	3
1001–10000	1	1	2	5
10001–and above	—	—	3	1

Table 4. *Chi-square values for categories of fish and prawn samples*

Category	No. of positive samples Fish	Prawn	Value of chi-square	Remarks
Incidence of *C. perfringens* on skin/shell	34	13	5.5	*
Count of *C. perfringens* on skin/shell				
Group I	24	0	16.03	***
Group II	10	13		
Incidence of *C. perfringens* in the intestine	16	11	8.54	**
Count of *C. perfringens* in the intestine				
Group I	7	7		
Group II	9	4	0.39	—

* significant at 5% level; ** significant at 1% level; *** significant at 0.1% level;

Enumeration of the total sulphite reducing clostridia was carried out by the method reported earlier (Lalitha & Iyer, 1986). All counts were expressed per 10 g of the sample. A standard MPN Table (FDA, 1978) was used to translate the number of enrichment cultures yielding *C. perfringens* into MPNs. The data were subjected to statistical analysis. The chi-square test was applied for the analysis of the data.

Results and Discussion

A total of 52 samples of fish and 14 samples of prawn were examined, the results of which are summarised in Tables 1 to 4.

Table 1 shows the distribution of *Clostridium perfringens* in fishes and prawns. *C. perfringens* was detected in 70 percent of the samples on the skin/shell surface. Only 40 percent of the samples showed presence of *C. perfringens* in the guts (Tables 2 & 3). 50 percent of the skin/shell samples had less than 100 per 10 g of *C. perfringens*. In 27 percent of the gut samples, the count exceeded 100 per 10g.

The frequency of isolation of *C. perfringens* from skin/shell and gut samples was tabulated in the form of (2 x 2) contigency table. The calculated chi-square value is 12.3 which is highly significant ($p < 0.001$). This clearly indicates that the frequency of isolation on the skin/shell is very much different from that of guts.

The frequency of isolation of *C. perfringens* from fish and prawn samples was calculated. The data were grouped into 4 categories and the calculated chi-square values (applied correction factor wherever necessary) were furnished in Table 4. In category 1, the chi-square value implies that the incidence of *C. perfringens* in fishes and prawns are different. In category 2, the levels of *C. perfringens* in various samples of the skin shell were grouped into 2: group I with very low level (count below 10 per 10 g) and group II, medium and high levels (count above 11 per 10 g) combined. The calculated chi-square value is highly significant and shows that the levels of *C. perfringens* (low and high) on the skin/shell as observed in fish are different in prawn samples. In category 3, the highly significant chi-square value indicates that incidence of *C. perfringens* in gut samples of fish is different from that of prawns. Incidence was high in prawns. As prawns inhabit the muddy bottom, they are better prone to contamination by the bacteria from the sediments. In category 4, the levels of *C. perfringens* in the guts of fish and prawn samples were grouped into 2; in group I, the count of *C. perfringens* ranged from 1–1000 and the count above 1001

was grouped as 2. The calculated chi-square value implies that the count of *C. perfringens* in the guts of fishes and prawns are similar.

Taniguti (1971) has reported that incidence of *C. perfringens* on the body surface is higher than that of guts in sea foods. A similar observation was noticed in this study also. Shrimps and other fishes inhabiting the bottom have high numbers of *C. perfringens*. Matches *et al.* (1974) had also made similar findings. Horsley (1977) had indicated that fish inhabiting in suspended sediment will become contaminated with bacteria from the sediment.

Bonde (1967) and Taniguti (1971) have observed variations in the distribution of *C. perfringens* in the intestine of fishes. Such variations were related to the habitat and feeding habits of fish. It was indicated earlier by Shewan & Hobbs (1967) that bacterial flora of fish is a reflection of their environment. Bacterial flora of the alimentary tract of fish reflect feeding habits (Horsley, 1977). In the present study, the count of *C. perfringens* on the skin as well as intestine was found to vary between species of fish and even between individuals with in a species.

In general, *C. perfringens* formed less than one percent of the total clostridial population on fish skin and 0.01 percent of the total clostridial population in the guts of fishes and prawns. Matches *et al.* (1974) has reported that the proportion of *C. perfringens* in the total clostridial population varied between 56 and 71 percent for sewage samples and only 0.4 to 4.1 percent for freshwater sediments and soil samples.

The authors are grateful to Shri M.R. Nair, Director, Central Institute of Fisheries Technology, Cochin for encouragement and facilities provided.

References

Beerens, H., Romond, C., Leepage, C. & Criquelion J. (1982) in *Isolation and Identification Methods for Food Poisoning Organisms*, (Janet E.L. Corry, Diane Roberts & F.A. Skinner, Eds.) p. 237, Academic Press, London

Bonde, G.J. (1967) *J. Water Pollution. Cont. Fed.* **39**, 45

Bryan, F.L. (1973) in *Microbial Safety of Fishery Products*, (Chichester C.O. & Gram, H.D., Eds.) p. 273, Academic Press, London

Burow, H. (1974) *Arch. Lebensmittel hyg.* **25**, 39

FDA (1978) *Bacteriological Analytical Manual*, 5th edn., Benjamin Franklin Station, Washington

Hauschild, A.H.W., Gilbert, R.J., Harmon, S.M., O'keeffe M.F. & Vahilefeld, R. (1977) *Can. J. Microbiol.* **23**, 884

Hobbs, B.C. (1979) in *Food Borne Infections and Intoxications*, (Hans Reimann & Frank, L. Bryan., Eds.) p. 131, Academic Press, London

Hobbs, B.C. & D. Roberts (1967) *Food Poisoning and Food Hygiene*, 5th edn., p. 43, Edward Arnold, London

Horsley, R.W. (1977) *J. Fish. Biol.* **10**, 592

Lalitha, K.V. & Iyer, K.M. (1986) *Fish. Technol.* **23**, 152

Matches, J. R., Liston, J. & Curran, D. (1974) *Appl. Microbiol.* **28**, 655

Nambiar, V.N. & Iyer, K.M. (1973) *Fish. Technol.* **10**, 6

Nichida, S., Seo, N. & Nakagawa, M. (1969) *Appl. Microbiol.* **17**, 303

Shewan, J. M. & Hobbs, G. (1967) in *Progress in Industrial Microbiology*, (Hockenhull, D.J.D., Ed.) p.169, Iliffe Books Ltd., London

Sohn, J.Y., Ryeom, K., Kim, Y., Lee, M., On, O. & Byu, J. K. (1973) *Repts. Korean Natll Inst. Health* **10**, 79

Taniguti, T. (1971) *Bull. Fac. Fish. Nagasaki Univ.* **31**, 1