Incidence of *Clostridium perfringens* in Fishes

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Fish collected from local landing centres and also from local markets were examined for the presence and enumeration of *Clostridium perfringens*. A medium described by Beerens *et al.*. (1982) was used for the detection and enumeration of *C. perfringens*. *C. perfringens* occurs in low numbers in fishes compared to prawns. Proper handling of fishes after landing can reduce the chance of any public health hazard by *C. perfringens*.

Clostridium perfringens, an important food poisoning organism is ubiquitous. This organism has been isolated from marine sediments from pollutted and non-polluted areas. Though not a member of the normal flora of fish, *C. perfringens* and other pathogens are known to contaminate the fish caught from polluted water (Guelin, 1952).

The occurrence of C. perfringens has been widely reported on fish (Taniguti, 1971; Sohn *et al.* 1973; Matches *et al.* 1974; Burow, 1974). Taniguti & Zenitani (1969) detected *C. perfringens* type A in a high percentage of the body surface of seafoods (fish and shellfish) examined from June to September, the isolation rate from the alimentary canal of fish and shellfish was low. Taniguti (1971) isolated *C. perfringens* type A from 65% of raw fish samples from retail shops in England. Bonde (1967) found high numbers of *C. perfringens* in the gut of plaice, flounder and mackerel but small to nil numbers in other species. Girard *et al.* (1979) found *C. perfringens* in the anaerobic skin culture of Hawaiian sardine *(Sardinella marquesensis)*. Nambiar & Iyer (1973) studied the incidence of *C. perfringens* in prawn canneries. Similar information on Indian fishes being scanty, an attempt was made to study the occurrence of *C. perfringens* in raw fishes and shellfishes.

Material and Methods

Random samples of fish were collected from the local landing centres and also from the local markets in and around Cochin. Samples were brought to the laboratory and analysed within 2–4 h.

Skin with muscle and intestine were analysed separately. About 10 g of the sample was aseptically removed and homogenised with 100 ml of sterile diluent having the following composition: g/l peptone-1, sodium chloride 5, pH 7.2. Decimal dilutions were prepared using 9 ml aliquots of the same diluent.

Most Probable Number method (3 tubes/dilution) using LS medium (Beerens *et al.* 1982) was followed for the enumeration of *C. perfringens*. The composition of the medium was as follows: trypsin-digested peptone from casein 5 g, yeast extract-2.5 g; sodium chloride 2.5 g; lactose 10 g; L-cysteine hydrochloride 0.3 g; distilled water 1000 ml. pH 7.1 ± 0.1 and sterilized at 115°C for 20 min. Just before use, the medium was boiled for 5 min, cooled and 0.5 ml of a 1.2% solution of anhydrous sodium metabisulphite and 0.5 ml of a 1% solution of ferric ammonium citrate were added to each tube containing 8 ml medium or 5 ml of the metabisulphite solution and 5 ml of ferric ammonium citrate solution to each flask containing 80 ml medium. Appropriate dilutions were added at the rate of 10 ml (using 90 ml of LS broth) 1 ml and 0.1 ml (using 9 ml of LS broth) and incubated 16–18 at 46°C ± 0.5°C. *C. perfringens* produces a black precipitate due to reduction of sulphite to sulphide and produces gas from lactose.

Simultaneous enumerations were carried out to determine the total aerobic count and also the total number of sulfite-reducing Clostridia. Total aerobic count was estimated in seawater agar having the following composition: peptone 10 g; agar 15 g;

a trace of ferric phosphate; dissolved in 1 litre aged seawater pH 7.2. Appropriate dilution was pour-plated and incubated aerobically at room temperature (29 ± 1°C) for 48 h and bacterial counts estimated. Total sulfite-reducing clostridial count was estimated in TS agar (Harmon *et al.* 1971) without egg-yolk and D-cycloserine. Appropriate dilution was pour-plated and incubated anaerobically (Spray, 1930) at 46°C for 24 h and black colonies were estimated.

Cultures from LS medium tubes showing positive growth were streaked on SC agar (Hauschild *et al.* 1974, 1977; ICMSF, 1978) and 5 colonies of presumptive *C. perfringens* were selected after an anerobic incubation of 24 h at 37°C. These colonies were inoculated into lactose-gelatin and nitrate-motility media (FDA, 1978) for confirmation.

Results and Discussion

Results obtained from examining fish samples by the preceding methods are presented in Tables 1, 2, 3 and 4. Table 1 presents the total aerobic count (TPC), total sulfite reducing clostridial count and *C. perfringens* count in the skin with muscle and intestine of oil sardines *(Sardinella longiceps)*. In fresh fish, TPC/g of skin with muscle ranged between 4.7 x 10^4 to 1.17 x 10^5 and that of intestine between 5.02 x 10^5 to 2.2 x 10^8. The total sulfite reducing clostridial count ranged between 1.1 x 10^2 to 1.04 x 10^3/g of skin with muscle samples and that of intestine between 1.07 x 10^3 to 3.40 x 10^4. *C. perfringens* was detected only once in the skin with muscle sample of fresh oil sardines. The count was 4.3/g. In the case of market samples, the count ranged between 0.4–11/g of the sample. In the intestine samples, there was not much difference in the counts between the fresh samples and the market samples. The count ranged between 0.7-2.4 x 10^2/g of the sample.

Table 2 indicates the total aerobic count, the total sulfite reducing clostridial count and *C. perfringens* count in the skin with muscle and intestine of mackerel. In fresh fish, total aerobic count ranged between 6.41 x 10^4 to 1.51 x 10^5/g of skin with muscle sample and that of intestine ranged between 3.00 x 10^6 to 4.23 × 10^7/g. The total sulfite reducing clostridial count in fresh fish

Table 1. *Bacterial loads on oil sardine*

Species		Source	Sample	TPC/g	Sulfite reducing *Clostridia*/g	*C. perfringens* cells/g
Sardinella longiceps	1	Landing centre	Skin with muscle	1.17 x 10^5	nd	nd
	2	,,	Intestine	5.02 x 10^5	1.07 x 10^3	2.4 x 10^2
	2	,,	SM	7.7 x 10^5	1.1 x 10^2	nd
			I	7.9 x 10^7	3.4 x 10^4	2.4 x 10^2
	3	,,	SM	4.7 x 10^4	1.04 x 10^3	4.3
			I	2.2 x 10^8	1.8 x 10^3	2.4 x 10^2
	4	Market	SM	1.32 x 10^6	2.94 x 10^3	9.3
			I	1.27 x 10^8	3.67 x 10^4	2.4 x 10^2
	5	,,	SM	1.29 x 10^6	4.04 x 10^3	11
			I	1.5 x 10^6	5.5 x 10^2	30
	6	,,	SM	4.4 x 10^6	1.71 x 10^5	0.4
			I	2.14 x 10^8	2.3 x 10^4	24
	7	,,	SM	1.47 x 10^6	1.13 x 10^3	1.5
			I	7.12 x 10^7	4.53 x 10^4	0.7
	8	,,	SM	3.37 x 10^6	2.1 x 10^3	1.1
			I	1.18 x 10^6	3.43 x 10^4	2.1

nd: not detected

Table 2. *Bacterial loads on mackerel (Rastrelliger kanagurta)*

Source	Sample	TPC/g	Sulfite reducing *Clostridia*/g	*C. perfringens*
Landing centre	Skin with muscle	1.51×10^5	5.8×10^3	nd
,,	Intestine	3.00×10^6	2.6×10^4	,,
	SM	6.41×10^4	1.73×10^3	,,
	I	4.23×10^7	2.75×10^4	,,
Market	SM	5.18×10^5	4.21×10^3	24
	I	1.6×10^7	1.43×10^4	nd
,,	SM	6.3×10^5	1.13×10^4	,,
	I	2.7×10^7	nd	,,
,,	SM	3.51×10^6	2.78×10^3	,,
	I	5.6×10^6	1.65×10^4	,,

nd: not detected

Table 3. *Bacterial loads on miscellaneous species*

Species	Source	Sample	TPC/g	Sulfite reducing *Clostrida*/g	*C. perfringens* cells/g
Lactarius lactarius	Landing centre	SM	6.7×10^5	9.06×10^4	2.1
		I	3.21×10^4	8.48×10^3	4
Ottolithus argenteus	Market	SM	4.37×10^5	2.82×10^3	0.9
		I	5.92×10^4	1.18×10^2	nd
		SM	5.71×10^5	3.62×10^3	24
		I	7.13×10^4	1.53×10^2	15
Mugil parsia	Market	SM	9.25×10^4	2.76×10^3	1.5
		I	1.31×10^5	1.03×10^3	nd
	,,	SM	1.02×10^5	3.13×10^3	0.4
		I	8.04×10^4	1.32×10^3	nd
Sillago sihama	Market	SM	3.56×10^5	2.34×10^2	nd
		I	7.63×10^4	3.37×10^2	24

nd: not detected

ranged between 1.73×10^3 to 5.8×10^3/g of skin with muscle sample and that of intestine between 2.6×10^4 to 2.75×10^4/g *C. perfringens* could not be detected in fresh mackerel samples. Of the five samples analysed, *C. perfrngens* was detected in the skin with muscle portion of 1 market sample. The count was 24/g.

The total aerobic count, total sulfite reducing clostridial count and *C. perfringens* count in the skin with muscle and intestine samples of four species of fishes are summarized in Table 3. In *Lactarius lactarius, C. perfringens* count was 2.1/g of skin with muscle and 4/g of intestine sample. In *Ottolithus argenteus, C. perfringens* count was 0.9–24/g of skin with muscle and that of intestine was 15/g. Of the two samples analysed, *C. perfringens* was detected only in 1 intestine sample. In the case of *Mugil parsia, C. perfringens* was detected only in the skin with muscle portions of the two samples analysed. The count was 0.4–1.5g. In *Sillago sihama, C. perfringens* was found to be present only in the intestine samples (24/g).

Table 4 summarises the total aerobic count, total sulfite reducing clostridial count

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