Incidence and Low Temperature Survival of *Salmonella* in Fishery Products*

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Salmonella was isolated from 12% of PD shrimps, 10% of HL shrimps, 14% of PUD shrimps, 17% of lobsters, 14% of cuttle fish, 25% of cat fish and 20% of seer fish (all frozen) tested. One percent of the fish meal, 4% of dried non-penaeid prawn and 23% of sea beach sand showed incidence of the organism. *Salmonella* was also isolated from 2 and 4% of the swab samples of utensil surfaces and the floor surface of the processing hall respectively as well as from 1% of the process water tested.

All the serotypes of *Salmonella* tested were resistant to freezing at –40°C, but during subsequent storage at - 20°C, there was some difference between the serotypes with regard to their viability, *S. paratyphi B* being the most resistant which survived upto 9 months while *S. saintpaul* the least resistant having survival upto 5 months only.

The marine fish collected from the open sea is free from *Salmonella* (Shewan, 1970; Bryan, 1973; Cann, 1977; Hobbs, 1982); but fishes from polluted coastal waters are reported to be contaminated with this organism (Shewan, 1962; Bryan, 1973; Zuberi & Qadri, 1981). Contamination of marine products with *Salmonella* from fishing boats (Morris *et al.*, 1970), untensils used for processing (Hobbs & Gilbert, 1970; Anderson *et al.*, 1971), food handlers (Lee, 1973; Bryan, 1973; Hobbs, 1974), process water (Morris *et al.*, 1970) and surroundings of the processing unit (Shewan, 1962) has been documented. The information available on the incidence of *Salmonella* in fishery products has been reviewed by Shewan (1962) and Bryan (1973). Review of literature indicates that no detailed study on the incidence of *Salmonella* in various fishery products has been carried out in India. The available information on the viability of *Salmonella* in fishery products during freezing and frozen storage is scanty and no meaningful inference can be drawn from the existing data. Detailed studies were, therefore, undertaken on the incidence and low temperature viability of *Salmonella* in different fishery products. The results of the study are summarised in this paper.

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Materials and Methods

All the samples examined in this study, except fresh fish and dried non-penaeid prawns (Jawala), were collected from the fish processing factories situated at Cochin. Fresh fish and dried Jawala prawns were from the retail markets in Bombay. About 50 g of fishery products were collected for bacteriological analysis. The swabbing technique of Tanner (1950) was followed to determine bacterial load on the fish-contact surfaces. Water and ice samples were collected in sterile bottles. The samples were transported to the laboratory in ice under aseptic conditions and were analysed normally within 3 hours after their collection.

Salmonella was isolated and identified as per the AOAC (1975). All the *Salmonella* strains isolated from different fishery products were examined and their viability during freezing and subsequent frozen storage was determined by inoculating them into sterile shrimp homogenates (20% w/w in water).

Aseptically centrifuged and washed suspension of young (24 h) bacterial cells were resuspended in 10 ml of sterile isotonic saline. The suspension was diluted 10^5 times using sterile normal saline. Based on the results of preliminary experiments, the bacterial suspension was added to the shrimp homogenate in appropriate quantities so as to get detectable numbers of bacteria in the final suspension. The inoculated homogenate taken in conical flasks was stirred for 15 min by using a magnetic stirrer and 50 ml of this was transferred to 100 ml conical flasks. The flasks were then kept in a freezer at –40°C for 2½ h. One flask from each strain was drawn, before and after freezing, as sample. The inoculated frozen shrimp homogenate was thawed by keeping the flask for 15 min in a water bath maintained at 45°C. At definite intervals, one flask from each strain was taken out of the deep freezer and thawed for detecting the organism as per the method cited above.

Results and Discussion

The incidence of *Salmonella* in various fishery products, fish-contact surfaces, water and ice is given in Table 1. The organism was found to be present in 2% of the swab samples of the surfaces of utensils tested. *Salmonella* could not be detected from the palms of workers. On the other hand, 4% of the swabs samples collected from the surface of the floor of the processing hall showed incidence of *Salmonella*. *Salmonella* was isolated from 1% of the water samples examined but not from any of the ice samples tested. In general, the incidence of *Salmonella* was found to be higher in fish samples compared to that in shrimps and cuttle fish. Twelve percent of the PD shrimps, 14% of PUD shrimps, 17% of frozen lobsters, 14% of cuttle fish, 25% of cat fish and 20% of seer fish showed incidence of *Salmonella*. No *Salmonella* was isolated from squids. The pathogen was also present in 1% of the fish meal, 4% of dried non-penaeid prawns and 23% of sea-beach sand.

Isolation of *Salmoncella* from 2% of the swab samples collected from the surfaces of utensils is highly significant. It is assumed that contamination of utensils with *Salmonella* might have taken place from the dropping of rodents and wall lizards. Incidence of *Salmonella* in the dropping of rodents (Jay, 1978) and wall lizards (Gupta *et al.*, 1980) is documented.

Salmonella was isolated from one of the 120 water samples tested in the present study. The Central Institute of Fisheries Technology has so far, analysed many hundreds of water samples for ascertaining its suitability for fish processing but no *Salmonella* has been isolated by the Institute from any of the samples. Therefore, the isolation of *Salmonella* in the present study could be viewed only as a stray case. On investigation it was found that the water tank was not covered and birds' dropping might have caused the contamination.

Table 1. *Incidence of Salmonella in various fishery products, fish-contact surfaces, water and ice*

Sample details	No. of samples analysed	No. of samples showing incidence of salmonella
Frozen shrimps:		
Headless shell-on	130	13 (10)
Peeled and deveined	150	18 (12)
Peeled undeveined	100	14 (14)
Cooked and peeled	180	0 (0)
Frozen lobsters:		
Headless shell-on	13	1 (17)
Frozen cuttle fish	50	7 (14)
Frozen squids	30	0 (0)
Frozen red snapper	1	1 (100)
Frozen cat fish	40	2 (25)
Frozen seer fish	40	8 (20)
Fresh fish	360	16 (4)
Dried non-penaeid shrimps (Jawala)	25	1 (4)
Fish meal	80	1 (1)
Poultry feed containing fish meal	1	1 (100)
Sea beach sand	22	5 (23)
Utensils	150	3 (2)
Palm of workers	50	0 (0)
Floor	50	2 (4)
Water	120	1 (1)
Ice	120	0 (0)

Percentages are given in parenthesis

In the present study, *Salmonella* has been isolated from all the fishery products except cooked frozen shrimps and squids. As marine fishes caught from the open sea are free from *Salmonella* (Shewan, 1962; Cann, 1977; Hobbs, 1982), the incidence of this organism in the products tested in the present study is likely to be the result of external contamination. Some of the existing commercial practices prevailing in the seafood industry seem to cause the contamination of the product with *salmonella*. For example, washing the catch with coastal sea water has often been reported to contaminate fish and shell fishes with *Salmonella* (Shewan, 1962; Cann, 1977; Zuberi & Qadri, 1981). Moreover, salmonella has often been isolated from Indian coastal environments (Gore *et al.*, 1980). In some parts of the country, the landed material is sorted out on the sea beach itself and this practice is likely to contaminate firhery products with *Salmonella*. The isolation of *Salmonella* from 23% of the sea beach sand in tbe present study supports this assumption. Another source of contamination is the floor of the processing hall, *Salmonella* has been isolated in the present study from 4% of the swab samples of the floor surface. Again from Table 1, it can be seen that 2% of the swab samples of the surfaces of utensils yielded *Salmonella* and thus fishery products on the processing line may get contaminated from this source also. Further, the isolation of *Salmonella* from one of the water sample tested, though an isolated case, when viewed from a strictly scientific angle, can be considered as an occasional source of contamination in the processing line.

Anderson *et al.* (1971) and Beckers *et al.* (1981) isolated salmonellae from cooked frozen shrimps; on the contrary, the organism was absent in all the cooked and peeled frozen shrimps in the present study. In general, the incidence of *Salmonella* was higher in frozen fishes compared to frozen shell fishes. This may be attributed to the difference in the methods of handling fish and shrimps and the extra care taken to process bacteriologically sound shrimps. There are no previous reports on the incidence of *Salmonella* in frozen cuttle fish, seer fish and red snapper.

The incidence of *Salmonella* in fish meal, poultry feed containing fish meal and dried non-penaeid prawns may be due to contaminated raw materials or lack of hygiene and sanitation during drying. There are previous reports on the incidence of *Salmonella* in fish meal (Anderson *et al.*, 1971; Hobbs, 1974; Hindawi & Taha, 1979).

The viability of *Salmonella* spp. in cooked shrimp homogenate at sub-zero temperatures is shown in Table 2. All the serotypes studied were resistant to freezing at – 40°C, but during subsequent storage at –20°C there was some difference between the sero-

Table 2. *Viability of Salmonella spp. in sterile shrimp homogenate during freezing at* - 40°C *and subsequent storage at* - 20°C

Serotype studied	Before freezing	After freezing	During cold storage on completion of 1st M	2nd M	3rd M	4th M	5th M	6th M	7th M	8th M	9th M	10th M
S. typhimurium	+	+	+	+	+	+	+	+	+	+	—	—
S. paratyphi B	+	+	+	+	+	+	+	+	+	+	+	—
S. newport	+	+	+	+	+	+	+	+	+	—	—	—
S. saintpaul	+	+	+	+	+	+	+	—	—	—	—	—
S. weltevreden	+	+	+	+	+	+	+	+	+	—	—	—
S. roan	+	+	+	+	+	+	+	+	+	—	—	—
S. bareilly	+	+	+	+	+	+	+	+	+	+	—	—
S. salford	+	+	+	+	+	+	+	+	+	+	—	—
S. way cross	+	+	+	+	+	+	+	+	+	+	—	—
S. poona	+	+	+	+	+	+	+	+	+	+	—	—

M = month; + = presence of growth; — = absence of growth
All the strains tested were isolated during the present study

types with regard to their viability. *S. paratyphi B* was the most resistant which survived upto 9 months while *S. saintpaul* was the least resistant having survival upto 5 months only. *S. typhimurium, S. bareilli, S. salford, S. waycross* and *S. poona* remained viable upto 8 months whereas *S. roan, S. newport* and *S. weltevreden* survived upto 7 months. Raj & Liston (1961) reported that *S. typhimurium* can survive for more than one year in fish homogenate held at – 17.9°C. According to Digirolamo *et al.* (1970), *S. typhimurium* and *S. derby,* suspended in pacific oysters, were sensitive to freezing and frozen storage at –17.8°C and at the end of 1 week of storage, 99.9% of the total number of salmonellae originally present lost their viability. The results of the present study are different from those of Raj & Liston (1961) and Digirolamo *et al.* (1970). Differences in the strains and the nature of substrates used may be the reasons for the difference in the results.

As, many of the *Salmonella* strains are resistant to freezing at –40°C and can remain viable for about 7 months during subsequent storage at – 20°C, all possible precautions should be taken to avoid contamination of fishery products with *Salmonella* by improving plant sanitation and hygiene and by adhering strictly to the technically accepted procedures for handling and processing the material.

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