On the Pattern of *Salmonella* Serotypes in Fishery Products, Froglegs and Processing Environments*

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Thirty four different serotypes of *Salmonella* have been isolated from aquatic products. The number of serotypes from frozen froglegs were 22. Only four serotypes were isolated from frozen shrimps. *S. weltevreden* predominates in frozen shrimps and fish. *S. roan* and *S. larochelle* were isolated for the first time in India. Isolation of six rare serotypes of *Salmonella* has also been reported.

Over 2000 Salmonella serotypes are known to-day and every year new serotypes are being added to the list. However, comparatively fewer number of serotypes only are encountered in any particular country. The National Salmonella and Escherichia Centre, Kasauli has reported the occurrence of 161 serotypes of Salmonella in India (Saxena, 1987 personal communication). The strains figured in this report were mostly isolated from human being, animals, water and sewage. Sharma *et al.* (1974), Rao & Nandy (1976), Andrews *et al.* (1977) and Rajagopalan (1978) have carried out brief studies on *Salmonella* serotypes in froglegs. But, no detailed study has so far been carried out anywhere on the pattern of occurrence of *Salmonella* serotypes in fishery products even though serological classification has been generally found to be useful in identifying the sources of contamination of processed products with *Salmonella*. Detailed studies were, therefore, carried out on the incidence of *Salmonella* pattern in fresh and frozen fishery products, froglegs and processing environments. The results of the study are presented in this paper.

Materials and Methods

Samples of the different fishery products (except fresh fish samples and dried jawala prawns) were collected from the processing factories situated at Cochin. The fresh fish samples and the dried jawala prawns were from the retail fish markets in Bombay. In addition, a few swab samples from fish contact surfaces, water and ice were also collected from the factories at Cochin. Details showing the number of samples from different sources are as follows:

Source	No. of samples
Frozen shrimps (Headless shell-on)	130
Frozen shrimps (peeled & deveined)	150
Frozen shrimps (peeled undeveined)	100
Frozen shrimps (Cooked and peeled)	100
Frozen lobsters (Headless shell-on)	13
Frozen cuttle fish	50
Frozen squids	30
Frozen red snapper	1
Frozen cat fish	8
Frozen seer fish	40
Frozen froglegs	120
Dried jawala prawns	150
Utensils	25
Floor	150
Worker's palm	50
Water	50
Ice	120
Raw shrimps	120
Raw fish	10
Raw lobsters	2
Raw froglegs	10

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Fish meal	80
Poultry feed containing fish meal	1
Sea-beach sand	20

Fishery products and the sea beach samples were collected in sterile containers. The swabs from contact surfaces were separately collected in 100 ml of lactose broth. Water and ice were collected in sterile bottles. The samples were transported to the laboratory under aseptic conditions. During transportation, the samples were kept cooled using crushed ice kept out of contact with the samples. In the laboratory, the samples were analysed for the presence of *Salmonella* as per the method recommended by AOAC (1975). The number of *Salmonella* strains isolated from different sources were as follows:

Frozen froglegs	165
Frozen shrimps	38
Frozen fish	30
Frozen lobsters	12
Raw froglegs	14
Raw shrimps	7
Raw fish	50
Raw lobsters	9
Fish meal	1
Poultry feed containing fish meal	7
Dried jawala prawn	7
Utensils	3
Floor of processing hall	5
Water	1
Sea beach sand	9
Total	358

The isolated strains were serotyped at the National Salmonella and Escherichia Centre at Kasauli (India) following the methods of Edwards & Ewing (1972) and Cowan (1972).

Results and Discussion

The salmonella serotypes isolated from different fishery products and related sources are given in Table 1. Thirty four different serotypes were isolated from these sources. The number of serotypes isolated from frozen froglegs were higher (22) compared to the number of isolates from other sources. Only four serotypes *(S. weltevreden, S. bareilly, S. orion* and *S. virchow)* were isolated from frozen shrimps. Eleven different serotypes were isolated from fresh fishes while six serotypes were isolated from frozen fish. The serotypes isolated from miscellaneous sources were 8; these included isolates from utensils, floor, water, sea-beach sand, raw froglegs, dried prawns, fish meal and poultry feed containing fish meal.

Normally, the incidence of *Salmonella* in marine fish and shellfish is the result of contamination from the unhygienic surroundings in which they are handled and processed. In the case of froglegs, on the other hand, it is due to contamination from its own intestine which is known to inhabit Salmonella (Iyer *et al.,* 1975). Recent studies have indicated the presence of a wide variety of *Salmonella* serotypes in the intestine of frogs (Sharma & Garg, 1976; Rajagopalan, 1978). Frogs are collected from paddy fields, canals, roadsides and similar habitats which are likely to get contaminated with sewage and faecal matter. Therefore, it is likely that the serotypes usually isolated from man and animals are isolated from frogs also. Saxena *et al.* (1983) have reported that, in India, *S. typhimurium, S. bareilly* and *S. saintpaul* are the most common serotypes isolated from human stool and that *S. saintpaul* and *S. typhimurium* are the dominating serotypes in animals and sewage respectively. Thus, the isolation of *S. typhimurium, S. saintpaul* and *S. bareilly* from frogs' intestine (Sharma & Garg, 1976; Rajagopalan, 1978) and from frozen froglegs in the present study are quite expected. *S. arizonae* was one of the major serotypes isolated from frozen froglegs (Table 1). *S. arizonae* has also been isolated by Sharma *et al.* (1974), Rao & Nandy (1976), Andrews *et al.* (1977) and Rajagopalan (1978) from frozen froglegs. *S. arizonae* is of reptile origin (Rowe, 1973) and as frogs live in almost the same natural habitats as that of reptiles, the isolation of this serotype from frozen froglegs is not unexpected. Plows *et al.* (1968) reported *S. arizonae* as the causative agent of gastroenteritis in Britain.

S. weltevreden was the dominating serotype in frozen shrimps, fresh fish, frozen fish, fresh lobsters and frozen lobsters (Table 1). In the case of fish and shrimps contamination with salmonellae appears to be mostly

due to unhygienic handling practices. *S. weltevreden* was isolated from the floor of the processing hall and from the utensils used in the processing factories (Table 1). Thus, it is likely that the floor and the utensils might have caused contamination of fish and shrimps with *S. weltevreden.* As rodents and wall lizards were seen in some of the processing units, it could be assumed that the utensils used in the processing units might have got contaminated from the droppings of rodents and wall lizards. In India, *S. weltevreden* has often been isolated from the intestinal contents of wall lizards (Kaura & Singh, 1968; Gupta *et al.,* 1980) and rodents (Kaura & Singh, 1968). Moreover, *S. weltevreden* has frequently been isolated from natural water sources (Basu *et al.,* 1975) also. It is, therefore, evident that *S. weltevreden* is usually present in the environment where fish, shrimps and lobsters are handled. Basu *et al.* (1975) have reported that, in India, infections due to *S. weltevreden* have increased both in man and animals after 1970.

The isolation of *S. larochelle* (6, 7 eh: 1,2) and *S. roan* 38, lv : enx (Table 1) have not been reported previously from any source in India. Later, 8 more isolations of *S. larochelle* were made in India and all these were from human sources: five from stool and three from urine (Saxena *et al.,* 1983). The role of *S. larochelle* in human salmonellosis is thus evident. The isolation of *S. cubana, S. waycross, S. stanley, S. paratyphi B* and *S. heidelberg* is reported for first time in frozen froglegs. *S. heidelberg* has rarely been isolated in India and only two isolations have been made previously – both from human sources (Saxena *et al.,* 1983). In India, till 1962, *S. cubana* had been isolated from human sources only. Later, a few isolations of this serotype were made from animals also, but still isolations from human sources predominate (Saxena *et al.,* 1984). *S. waycross,* isolated from frozen froglegs, is also a rare serotype and only one strain has, so far, been isolated in this country *(Saxena et al.,* 1980). This serotype has not been isolated from human sources. Saxena *et al.* (1983) have reported the isolation of this serotype from the intestines of frogs and therefore, contamination of froglegs from this source is quite possible. In India, *S. saintpaul* and *S. bareilly* have been isolated from many sources like human, cattle, pig, poultry, frog's intestine, laboratory animals, water and sewage (Saxena *et al.,* 1983). The wide distribution of these serotypes explains their presence in many of the products tested in the present study. Amongst the various serotypes isolated in India, *S. bareilly* and *S. saintpaul* occupy the 3rd and 4th position in the order of predominance (Saxena *et al.,* 1984). *S. bareilly* has been isolated from the floor of the processing hall (Table 1). Ghosh *et al.* (1960) have reported an outbreak of food poisoning in Calcutta (India) due to *S. newport. S. newport, S. chingola, S. anatum* and *S. bredeney* have been isolated from frozen froglegs only (Table 1). In India, all these serotypes have been isolated from natural waters and sewage (Saxena *et al.,* 1983). Two strains of *S. poona* have been isolated in the present study, one each from frozen fish and raw shrimps. In India, *S. poona* has been isolated from human sources only (Saxena *et al.,* 1988). Contamination of seafoods with *S. poona* from human sources is, therefore, suspected. *S. typhimurium* was isolated from one sample of water used for shrimp processing. In India, though *S. typhimurium* has frequently been isolated from natural waters and sewage (Saxena *et al.,* 1983), there is no previous record of isolation of this serotype from the water used by the seafood industry. As bird's droppings were seen inside the overhead watertank, contamination from this water source is quite likely.

In the present study, *S. alachua, S. muenchen* and *S. eastbourne* were isolated from raw fish. As per the report of the Centres for Disease Control (1981) of the U.S., *S. alachua* is to be classified as an uncommon and rare serotype. In India, this serotype was first isolated at Bombay by Das and Jayaraman (1955) from fowls. Dewan *et al.* (1976) have reported the isolation of *S. alachua* from sewage and water sources in India. Till recently, this serotype was thought to be mainly confined to animal sources. But, Chaudhuri *et al.,* (1978) reported the isolation of *S. alachua* from human sources and pointed out its association with clinical morbidity and mortality.

According to the latest compilation on the pattern of the occurrence of *Salmonella* in India, *S. muenchen* has not been isolated from human sources in this country whereas

Table 1. *Salmonella serotypes isolated from different fishery products and related sources*

Serotype	Number of strains isolated							
	Frozen froglegs	Raw fish	Frozen fish	Raw shrimps	Frozen shrimps	Raw lobsters	Frozen lobsters	Other sources
S. tyhimurium	26 (15.7)	—	3 (10)	1 (14.2)	—	1 (11)	1 (8.3)	2[a] (4.2)
S. arizonae	20 (12.1)	—	2 (2.6)	—	—	—	—	—
*S. roan**	18 (11.0)	—	—	—	—	—	—	6[b] (12.7)
S. saintpaul	12 (7.2)	—	—	1 (14.2)	—	—	—	2[c] (4.2)
S. bareilly	11 (6.6)	2 (4)	3 (10)	—	2 (5.2)	—	—	16[d] (34.5)
S. newport	11 (6.6)	—	—	—	—	—	—	—
S. anatum	10 (6.1)	—	—	—	—	—	—	—
S. bredeney	6 (3.6)	—	—	—	—	—	—	—
*S. matopeni***	6 (3.6)	—	—	—	—	—	—	—
S. chester	5 (3.1)	—	—	—	—	—	—	—
*S. nchanga***	5 (3.1)	—	—	—	—	—	1 (8.3)	—
*S. chingola***	5 (3.1)	—	—	—	—	—	—	—
S. hvittingfoss	5 (3.1)	—	—	—	—	—	—	—
*S. heidelberg***	5 (3.1)	—	—	1 (14.2)	—	—	—	—
*S. waycross***	4 (2.4)	—	—	—	—	—	—	—
S. stanley	3 (1.8)	3 (6)	—	—	—	—	—	—
S. richmond	3 (1.8)	—	—	—	—	—	—	1[e] (2.1)
S. salford	3 (1.8)	—	—	—	—	—	—	—
S. virchow	2 (1.2)	6 (12)	—	2 (29)	1 (2.6)	—	—	—
*S. larochelle**	2 (1.2)	3 (6)	—	—	—	—	—	—
Rough strain	2 (1.2)	—	—	—	—	—	—	—
S. cubana	1 (0.6)	—	—	—	—	—	—	—
S. senftenberg	—	—	—	—	—	—	3 (25)	—
*S. orion***	—	—	1 (3.4)	—	1 (2.6)	—	—	—
S. weltevreden	—	16 (32)	20 (66.6)	1 (14.2)	34 (89.6)	6 (89)	7 (58.4)	6[f] (12.7)
S. poona	—	—	1 (3.4)	1 (14.2)	—	—	—	—
S. paratyphi B	—	—	—	—	—	—	—	7[g] (14.8)
S. ohio	—	2 (4)	—	—	—	—	—	—
S. muenchen	—	4 (8)	—	—	—	—	—	—
S. alachva	—	4 (8)	—	—	—	—	—	—
*S. eastbourne***	—	2 (4)	—	—	—	—	—	—
S. oranienberg	—	7 (14)	—	—	—	—	—	—
S. subgenus III	—	1 (2)	—	—	—	—	—	—
S. enteritidis	—	—	—	—	—	—	—	7[h] (14.8)

* Isolated for the first time in India; ** Rarely isolated serotype in India.

Figures in parenthesis indicate the percentage occurrence of the corresponding serotype amongst the total number of Salmonella strains isolated from the respective source.

2[a] — one strain from water and one strain from floor

6[b] — all the strains are from raw froglegs

2[c] — both the strains are from raw froglegs

16[d] — six strains from poultry feed, three strains from floor and seven strains from sea-beach sand

1[e] — from raw froglegs

6[f] — one strain from floor, three strains from nutensils, and two strains from sea-beach sand

7[g] — five strains from raw FL, one strains from fish meal and one strain from poultry feed

7[h] — all strains are from dried jawala prawns

31 strains have been isolated from animals (Saxena *et al.*, 1980). In the U.S., on the other hand, *S. muenchen,* in 1978, occupied 14th position in the list of the 20 most frequently reported *Salmonella* serotypes from human sources (Centres for Disease Control, 1981). Morris *et al.* (1970) reported *S. muenchen* as one of the serotypes commonly isolated from fish meal during 1962–64. In Iraq, Nassir Al Hindawi *et al.* (1979) have reported the presence of *S. muenchen* in 2 out of the 30 fish powder samples analysed by them. In the U.S., *S. muenchen* is seldom reported to be present in estuary waters and shell fish (Metcalf *et al.,* 1973). In Australia, *S. muenchen* was one amongst the more frequently isolated strains from the cases of human salmonellosis during 1967–71 (Suttoon, 1973). An epidemic among infants caused by *S. muenchen* has been reported by Silverstolpe *et al.* (1961). Thus the pathogenic role of *S. muenchen* is quite evident.

S. eastbourne is a rarely isolated serotype in India, as only 5 strains of this serotype have so far been isolated in the country within a period of 24 years (Iyer, 1985). 4 strains have been isolated from sewage in Nagpur and one was isolated from frozen shrimps in Madras. There is no previous record of isolation of this serotype from fin fish. In the U.S. also, this serotype has been classified as an uncommon and rare serotype (Centres for Disease Control, 1981).

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