

Shigella in Fish and Fishery Products and its Survival in Prawn Homogenate at -20°C

S. SANJEEV and M.K. MUKUNDAN
Central Institute of Fisheries Technology
P.O. Matsyapuri, Cochin - 682 029, India

Four hundred and thirty-three samples consisting of fresh fish and frozen fish products collected from markets and fish processing factories from Cochin were examined for the incidence of enteric pathogen, *Shigella*. All samples were found to be free from *Shigella*. Survival of different pathogenic species of *Shigella* viz., *S. dysenteriae* (ATCC-2335), *S. flexneri* (ATCC-12022) and *S. sonnei* (ATCC-11060) in prawn homogenate stored at -20°C was studied. *S. dysenteriae* and *S. sonnei* could survive only up to 35 days but *S. flexneri* could survive for more than 52 days.

Key words : Fish, fish products, *Shigella dysenteriae*, *Shigella sonnei*, *Shigella flexneri*

Shigella constitute a genus of Gram-negative, non-motile, non-spore forming, rod-shaped bacteria with four species or serotypes, namely *S. boydii*, *S. dysenteriae*, *S. flexneri* and *S. sonnei* (Holt *et al.*, 1994). Virulent *Shigella* organisms cause the human illness known as bacillary dysentery. *S. sonnei* is encountered in industrialized nations, whereas *S. dysenteriae*, *S. flexneri* and *S. boydii* are largely confined to developing countries (Mehlman *et al.*, 1985). Although, mortality rates associated with shigellosis are relatively low in the United States (0.1%) (Bean & Griffin, 1990) they are considerably higher in developing countries, particularly for young children (Guerrant, 1985; Taylor *et al.*, 1989). Epidemiological studies on *Shigella* have established that 10 cells are sufficient to be an infective dose (Wachsmuth & Morris, 1989). *Shigella* spp. have been considered waterborne disease organisms. However, shigellosis outbreaks have been traced to contaminated chicken, fish, seafood, vegetables and fruits (Taylor & Nakamura, 1964; Black *et al.*, 1978; Bryan, 1979; Smith, 1987; Davis *et al.*, 1988).

Shigella is present in stools of infected persons. The contamination of lakes, rivers, ponds and seas with excreta of these patients leads to contamination of fish and seafood with this organism. Information regarding the incidence of *Shigella* in fish and fish product is very little. Similarly,

no data is available about its survival rate in fish substratum at cold storage temperature i.e., -20°C. Hence, the present study was taken up to study these aspects.

Materials and Methods

Surveillance of Shigella in fish and fish products

Four hundred and thirty-three samples consisting of fresh finfish, fresh shellfish, iced shellfish and frozen fishery products (Table 1) collected from markets and fish processing plants situated in and around Cochin were examined for the incidence of *Shigella* as per the method of International Commission on Microbiological Specification for Foods (ICMSF, 1978).

Survival of Shigella in prawn homogenate stored at -20°C

Prawn homogenate was prepared by homogenizing the required quantity of cooked prawns in normal saline (25% w/v) and 10 ml of the suspension was then transferred into test tubes and sterilized at 15 lbs for 30 min.

Type cultures of *S. dysenteriae* (ATCC-2335), *S. flexneri* (ATCC-12022) and *S. sonnei* (ATCC-11060) were used for the survival studies. The three species of *Shigella* were inoculated into separate tubes containing 10 ml sterile brain heart infusion broth (Difco) and incubated at 37°C for 24 h. The broth culture was then centrifuged for 15 min at 5000 rpm and the supernatant was aseptically decanted off. The cells were washed twice using sterile normal saline. Finally, the cell mass was resuspended in 10ml fresh sterile normal saline. The suspensions were diluted 10 times using sterile normal saline. This served as the stock inoculum. A 100µl cell suspension of each *Shigella* culture from the stock inoculum were inoculated into 3 sets of sterile prawn homogenate, mixed well and stored in a deep freezer (-20°C).

Immediately after inoculation and mixing, 1 ml of the homogenate from each set was plated separately using tryptone glucose beef extract agar (TGBEA) to find out the initial load of the organisms. The plates were incubated at 37°C for 48 h and the colonies were counted. One tube from each set kept at -20°C was taken out periodically and plated as described above.

Results and Discussion

Isolation of *Shigella* from fishes collected from sea, estuary and freshwater, especially prawns and mussels have been reported by several

authors (Dartville & Desmet, 1975; Lam & Hwee, 1978; Cantoni *et al.*, 1980; Singh & Kulshrestha, 1993; Singh, 1996). However, during the current study, *Shigella* species were not detected in any of the 433 samples of fish and fishery products collected from Cochin (Table 1). Varma & Iyer (1987) also have reported the absence of *Shigella* in 118 samples of frozen fish products collected from this area.

Table 1. Details of fish and fishery products tested

Name of sample	No. of samples tested
Fresh finfish	145
Fresh shellfish	45
Frozen prawns	51
Frozen squid	31
Frozen cuttlefish	23
Frozen crab	10
Frozen octopus	7
Frozen finfish	4
IQF prawns	40
IQF squid	12
Dried fish	35
Iced prawns	7
Iced squid	11
Cooked clams	12
Total	433

Table 2. Survival of *Shigella* spp. in prawn homogenate stored at - 20°C

Days of storage	<i>S. dysenteriae</i> cfu.ml ⁻¹	<i>S. flexneri</i> cfu.ml ⁻¹	<i>S. sonnei</i> cfu.ml ⁻¹
0	1.2x10 ⁵	1.4x10 ⁶	5.5x10 ⁵
2	1.3x10 ³	6.8 x10 ⁴	1.4x10 ⁴
5	1.2x10 ³	4.8x10 ⁴	6.8x10 ³
9	1.0x10 ³	6.5x10 ³	3.9x10 ³
16	6.0x10 ²	1.5x10 ⁴	5.0x10 ²
21	4.8x10 ²	2.2x10 ⁴	5.9x10 ²
32	80	3.6x10 ³	Nil
40	Nil	260	Nil
52	Nil	10	Nil
72	Nil	Nil	Nil

Survival of *S. dysenteriae* (ATCC-2335), *S. flexneri* (ATCC-12022) and *S. sonnei* (ATCC-11060) in prawn homogenate stored at -20°C are shown in Table 2. The studies revealed that *Shigella* spp. could not survive even up to 2 months at -20°C. *S. dysenteriae* and *S. sonnei* could survive only up to 35 days. When inoculated into foods in high numbers, *Shigella* species may survive for periods ranging from less than 3 weeks to more than 3 months in foods like stewed apples, cheese, flour, milk, seafood, eggs, tomato juice, cooking oil, root beer and ginger ale (Morris, 1984). *Shigella* may survive in water up to 120 days (Wachsmuth & Morris, 1989).

The studies have shown that all the fish and fish products examined were free from the enteric pathogen *Shigella* and that the pathogen could not survive in prawn substratum at frozen condition (-20°C) for more than 2 months. The results further indicate that *Shigella* is not a serious problem with regard to our fish and fish products.

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