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Incidence of Pathogenic Halophilic *Vibrios* in Frozen Fish Products

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Three hundred and eighty two samples consisting of iced and frozen fish products were collected from 23 processing factories situated in Kerala and Tamil Nadu for studying the incidence of pathogenic halophilic *Vibrios*. Halophilic *Vibrios* were isolated from 193 samples (50.52%). 171 samples (44.76%) were found to be the carriers of pathogenic halophilic Vibrios. Nine species of pathogenic halophilic *Vibrios* and nine species of non-pathogenic halophilic *Vibrios* were isolated from these samples. Among pathogenic organisms, *Vibrio cincinnatiensis* was found to be the dominant species (18.06%) followed by *Vibrio alginolyticus* (15.18%), *Vibrio parahaemolyticus* (9.42%), *Vibrio vulnificus* (5.24%) etc. *Vibrio harveyi* was the major species among non-pathogenic halophilic *Vibrios* (12.30%).

Key words: Halophilic *Vibrios*, pathogenic *Vibrios*, frozen products, iced products

Halophilic *Vibrios* are one of the major groups of bacteria found in marine and estuarine environments. It is therefore to be expected that freshly harvested finfish and shellfish from these environments might be harbouring some of the pathogenic halophilic *Vibrio* spp. *Vibrios* may cause a variety of diseases including gastroenteritis, wound infection, ear infection and primary and secondary septicemia. Association of *Vibrio* spp. with different clinical symptoms has been reported by Pavia *et al.* (1989). The *Vibrio* spp. related to wound infection, ear infection etc. would be a hazard for seafood handlers. *V. cholerae* and *V. mimicus* are not halophilic *Vibrios*. Most of the infections with halophilic *Vibrios* are known to be associated with either consumption of seafood or exposure to marine environments.

Infections due to *V. parahaemolyticus* is a widely occurring phenomenon (Blake *et al.*, 1980 and Joseph *et al.*, 1982) and is the main cause of summer diarrhoea in Japan (Zen-Yoji *et al.*, 1965). *Vibrio vulnificus* infection, which causes septicemia and soft tissue necrosis was first recognized in the United States (Hollis *et al.*, 1976), and sporadic cases have been reported in Japan (Matsuo *et al.*, 1978), Australia (Ghosh & Bowen, 1980), Belgium (Mertens *et al.*, 1979) and in Hong Kong (Woo *et al.*, 1984). *Vibrio hollisae* and *Vibrio furnissii* have been implicated in several cases of food poisoning (Hackney & Dicharry, 1988) and it is believed that *Vibrio fluvialis* may cause diarrhoea (Huq *et al.*, 1980).

A number of studies on Vibrios in seafoods have been reported (Kaneko & Colwell., 1973; Tison *et al.*, 1986; Kaysner *et al.*, 1987a, 1987b; O' Neill *et al.*, 1990; Matte *et al.*, 1994a; Chan *et al.*, 1989). In India, however, informations about the distribution of halophilic pathogenic *Vibrios* in seafood is scarce, or has been directed mainly towards *V. parahaemolyticus*. Some consignments of frozen fish exported from this country suffered rejection due to the presence of pathogenic halophilic *Vibrios* mainly *V. parahaemolyticus* and *V. vulnificus*. At present we have no information regarding the incidence of this group of organisms in frozen fish products meant for export. The aim of these investigations was to study the distribution of pathogenic halophilic *Vibrios* in iced and frozen fish and fish products meant for export.

Materials and Methods

Three hundred and eighty two samples consisting of iced and frozen fish and fish

products collected from 23 fish processing factories situated in Kerala and Tamil Nadu meant for export were examined for the incidence of halophilic *Vibrios* by the method given by FDA (Elliot, *et al.*, 1995). Isolates were identified as per the key proposed by Alsina & Blanch (1994).

Results and Discussion

The incidence of halophilic *Vibrios* and pathogenic halophilic *Vibrios* in the samples analysed is given in Table 1. Halophilic *Vibrios* were isolated from 50.52% of the samples and pathogenic halophilic *Vibrios* were isolated from 44.76% of the samples. Incidence of pathogenic halophilic *Vibrios* were found to be high in frozen split crab and IQF squid (100%), followed by iced squid (94.44%), iced prawns (86.21%) and it was minimum in cooked IQF prawns (15%).

Distribution of various species of pathogenic and non-pathogenic halophilic *Vibrios* in the samples studied is given in Table 2 and Table 3. Among pathogenic halophilic *Vibrios*, *V. cincinnatiensis* was found to be the dominant species (18.06%) followed by *V. alginolyticus* (15.18%), *V. parahaemolyticus* (9.42%), *V. vulnificus* (5.24%) etc. Single species of pathogenic halophilic *Vibrio* was detected in 26.18% of the samples. More than one species of pathogenic halophilic *Vibrio* per sample were also observed. Pathogenic halophilic *Vibrios* were absent in 55.24% of the samples. *V. harveyi* was the dominant species among non-pathogenic halophilic *Vibrios* (12.30%).

Karunasagar *et al.*, (1990) reported that *V. parahaemolyticus* was the most commonly encountered halophilic pathogenic *Vibrio* followed by *V. vulnificus* in market samples of fish and shellfish of Karnataka State. Prasad & Rao (1994) studied the distribution of pathogenic *Vibrios* in fresh, iced and frozen prawns and fish of Kakinada coast and reported the incidence of halophilic *Vibrios*, viz. *V. parahaemolyticus, V. vulnificus, V. metschnikovii, V. anguillarum* and Group F Vibrios. Thampuran *et al.*, (1996) isolated pathogenic halophilic *Vibrios* viz. *V. alginolyticus, V. parahaemolyticus, V. vulnificus, V. cincinnatiensis, V. damsela* and *V. metschnikovii* from coastal waters and fishes of Cochin. This was probably the first study on the pathogenic halophilic *Vibrios* from fish products meant for export from this country.

Table 1. Incidence of halophilic *Vibrios* and pathogenic halophilic *Vibrios* in seafood samples

Name of Sample	Total No. of sampe tested	Samples with halophilic *Vibrios*		Samples with pathogenic halophilic *Vibrios*	
		Number	% of total	Number	% of total
Frozen fish fillets	13	8	61.54	7	53.85
Frozen cuttle fish	29	12	41.38	8	27.59
IQF prawns	100	17	17.00	15	15.00
Frozen prawns (block)	68	39	57.35	34	50.00
Frozen squid	84	40	47.62	36	42.86
Frozen octopus	14	7	50.00	6	42.85
Frozen ark shell	4	2	50.00	2	50.00
Frozen split crab	6	6	100.00	6	100.00
IQF squid	6	6	100.00	6	100.00
Frozen yellow clams	5	5	100.00	4	80.00
Iced prawns	29	29	96.55	25	86.21
Iced squid	18	18	100.00	17	94.44
Iced oyster	6	5	83.33	5	83.33
Total	382	193	50.52	171	44.76

Table 2. Occurrence of various species of pathogenic halophilic *Vibrios* in different fish products.

Name of Sample	Total No. of samples	Number of samples carrying *Vibrio* species							
		V. alginolyticus	*V. carchariae*	*V. cincinatiensis*	*V. damsela*	*V. fluviali*	*V. furnissii*	*V. metschnikovii*	*V. parahaemolyticus*	*V. vulnificus*
Frozen fish fillets	13	2	1	Nil	Nil	1	2	Nil	1	Nil
Frozen cuttle fish	29	4	Nil	7	Nil	Nil	Nil	Nil	Nil	Nil
IQF prawns	100	4	2	4	Nil	1	Nil	2	1	2
Frozen prawns (block)	68	10	Nil	18	Nil	Nil	Nil	6	8	6
Frozen squid	84	17	Nil	13	Nil	2	Nil	2	4	6
Frozen octopus	14	Nil	Nil	3	Nil	1	1	Nil	2	1
Frozen ark shell	4	Nil	Nil	1	Nil	Nil	Nil	Nil	1	Nil
Frozen split crab	6	3	Nil	3	Nil	Nil	Nil	Nil	Nil	Nil
IQF squid	6	1	1	3	Nil	1	1	1	Nil	Nil
Frozen yellow clams	5	3	1	1	Nil	Nil	Nil	Nil	2	1
Iced prawns	29	7	Nil	6	1	2	Nil	1	10	1
Iced squid	18	6	2	10	Nil	Nil	Nil	Nil	5	1
Iced oyster	6	1	Nil	Nil	Nil	Nil	Nil	Nil	2	2
Total	382	58	7	69	1	8	4	12	36	20
%		15.18	1.83	18.06	0.26	2.09	1.05	3.14	9.42	5.24

V. parahaemolyticus has been implicated in more than 1000 outbreaks of bacterial food poisioning per year in Japan and it accounts for 45-70% of total incidence of this nature in that country (Hackeney & Dicharry, 1988). *V. parahaemolyticus* has also caused wound infections, ear infections and rarely secondary septicemia (Blake, 1984). Raw seafood is the usual source of contamination. In the U.S., most of the foods implicated in *V. parahaemolyticus* outbreaks are cooked seafoods that have been recontaminated, although raw oyster and raw crabs had been implicated in some outbreaks (Barker 1974; Spite *et al.*, 1978; Blake *et al.*, 1980).

The incidence of *V. parahaemolyticus* in marine finfish and shellfish varied from 67 to 92% (Sanjeev & Stephen 1993; Matte *et al.*, 1994, b) but in fish products it was less i.e. 3.69 to 30.23% (Sanjeev & Stephen, 1994; Sunen *et al.*, 1995). In the present study it was observed that the incidence of *V. parahaemolyticus* in fish products was 9.42%.

V. vulnificus is one of the most invasive species ever described (Oliver, 1985). This

Table 3. Occurrence of various species of non-pathogenic halophilic *Vibrios* in different fish products.

Name of Sample	Total No. of samples	Number of samples carrying *Vibrio* species								
		V. anguillarum	*V. campbelli*	*V. harveyi*	*V. logei*	*V. marinus*	*V. mediterranei*	*V. orientalis*	*V. pelagius*	*V. splendidus*
Frozen fish fillets	13	Nil	Nil	1	Nil	1	2	Nil	1	Nil
Frozen cuttle fish	29	4	Nil	4	Nil	Nil	Nil	Nil	1	3
IQF prawns	100	2	1	Nil	2	Nil	1	2	Nil	4
Frozen prawns (block)	68	5	3	5	Nil	Nil	Nil	2	Nil	2
Frozen squid	84	2	Nil	6	Nil	2	Nil	Nil	3	2
Frozen octopus	14	1	Nil	2	Nil	Nil	Nil	Nil	Nil	1
Frozen ark shell	4	Nil	Nil	1	Nil	Nil	Nil	Nil	Nil	Nil
Frozen split crab	6	Nil	Nil	2	Nil	Nil	1	Nil	Nil	Nil
IQF squid	6	Nil	Nil	1	Nil	Nil	Nil	Nil	Nil	Nil
Frozen yellow clams	5	Nil	Nil	2	1	Nil	Nil	Nil	Nil	Nil
Iced prawns	29	Nil	3	5	1	Nil	Nil	1	2	2
Iced squid	18	Nil	2	18	Nil	Nil	Nil	2	Nil	Nil
Iced oyster	6	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1
Total	382	14	9	47	4	2	2	7	6	15
%		3.66	2.36	12.30	1.05	0.52	0.52	1.83	1.57	3.93

organism is phenotypically similar to *V. parahaemolyticus* and has been identified as an etiological agent for three syndromes, primary septicemia, skin infection and acute diarrhoea. *V. vulnificus* infection and primary septicemia are not uncommon in the United States, and many of the wound infections lead to second septicemia (Blake, 1984). The incidence of *V. vulnificus* in water samples and bivalves in some countries has been reported to be about 19 percent (O' Neil *et al.*, 1990). Apparently there is no other information regarding the incidence of *V. vulnificus* in fish products for comparison.

V. hollisae has been implicated in several cases of food poisoning. Symptoms include diarrhoea and in approximately half the cases, vomiting and fever. Seafood is implicated as the vehicle for *V. hollisae* including raw oyster, clam and shrimp (Morris *et al.*, 1982). All the samples analysed during this investigation were found to be free from this pathogen.

V. fluvialis was found to be present in about 2% of the samples. This organism has been recognised as a potential pathogen since 1971 (Morris & Black, 1985). In Brazil it was detected in cases of infant diarrhoea (Mugalhaes *et al.*, 1990).

Other pathogenic halophilic *Vibrios*, viz. *V. alginolyticus, V. damsela, V. cincinnatiensis, V. carchariae* and *V. metschnikovii* are not commonly associated with food borne diseases but are implicated in wound infections, ear infections, meningitis etc. *V. alginolyticus* has also been reported to be the cause of diarrhoeal diseases in human beings consuming contaminated fish (Blake *et al.*, 1980). It is generally accepted that *V. alginolyticus* is present in sea water and fresh marine fishes in larger numbers than other *Vibrio* spp. (Chan *et al.*, 1986). In the present study it was observed that the incidence of *V. cincinnatiensis* was more frequent than *V. alginolyticus* in frozen fish products.

These results should be of concern to the food and public health officials and clinical bacteriologists. Hence, it may be concluded that the presence of various pathogenic halophilic *Vibrio* in frozen fish products meant for export should be viewed seriously in the light of vast export potential.

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