

Quantitative Studies on Halophilic Pathogenic Vibrios in Fish and Fish Products

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One hundred and thirty samples consisting of iced, block frozen and individually quick frozen (IQF) fish and fish products collected from landing centres, markets and fish processing factories situated in and around Cochin were examined for the incidence of halophilic pathogenic vibrios by using MPN method. Halophilic pathogenic vibrios were isolated from 72% of iced samples, 40.4% of block frozen samples and 29.2% of IQF samples. In general, 51.5% of the samples was found to be positive for halophilic pathogenic vibrios. *Vibrio cincinnatiensis* was found to be the dominant species isolated from different fish and fish products (incidence 30%, load varied from <1- 930 MPN g⁻¹) followed by *V. alginolyticus* (25.4%, <1-360 MPN g⁻¹), *V. vulnificus* (16.9%, <1- 530 MPN g⁻¹), *V. parahaemolyticus* (8.5%, <1-9.1 MPN g⁻¹), *V. metschnikovii* (4.6%, <1-30 MPN g⁻¹), *V. furnissii* (2.3%, <1-30 MPN g⁻¹), *V. fluvialis* (0.8%, <1 MPN g⁻¹) and *V. damsela* (0.8%, <1- 3.6 MPN g⁻¹). IQF samples showed the lowest incidence of halophilic pathogenic vibrios. Considering the seafood export potential of India, incidence of halophilic pathogenic vibrios in fish and fish products is a matter of serious concern.

Key words: Quantitative studies, halophilic pathogenic vibrios, fish and fish products

Halophilic vibrios are one of the major groups of gram-negative bacteria indigenous to marine and estuarine waters. It can be detected only in warm seasons in the temperate zone (Liston & Baross, 1973). They are expected to be prevalent throughout the year in the tropical zone (Elhadi *et al.*, 2004). Freshly harvested finfish and shellfish from these environments harbour some of the halophilic vibrios. Halophilic pathogenic vibrios may cause a variety of diseases including gastroenteritis, wound infection, ear infection and septicaemia. Association of *Vibrio* spp. with different clinical symptoms has been reported by Pavia *et al.* (1989). Currently 10 halophilic *Vibrio* spp. viz., *V. alginolyticus*, *V. carchariae*, *V. cincinnatiensis*, *V. damsela*, *V. fluvialis*, *V. furnissi*, *V. hollisae*, *V. metschnikovii*, *V. parahaemolyticus* and *V. vulnificus* are known to cause or to be associated with human infections (Kelly

et al., 1991, Dalsgaard *et al.*, 1996). The *Vibrio* spp. related to wound infection and ear infection would be a hazard for seafood handlers while others would be a hazard for seafood consumers. Incidence of these species in seafood is therefore of public health significance.

Incidence of halophilic pathogenic vibrios in a variety of seafoods has been reported (Neumann *et al.*, 1972; Kaneko & Colwell, 1973; Molitoris *et al.*; 1985; Tison *et al.*, 1986; Kaysner *et al.*, 1987, O'Neill *et al.*, 1990; Sanjeev & Stephen, 1993; Prasad & Rao, 1994, Sunen *et al.*, 1995; Thampuran & Surendran, 1996; Sanjeev *et al.*, 2000; Health *et al.*, 2002; Elhadi *et al.*, 2004; Parisi *et al.*, 2004). However, information regarding the load of halophilic pathogenic vibrios in seafood is scarce, or has been directed mainly towards *V. parahaemolyticus*. Some

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consignments of frozen seafood exported from India suffered rejection due to the presence of *V. parahaemolyticus* and *V. vulnificus* (Varma *et al.*, 2003). Microbial quality defects were also reported by importing countries due to the detection of other halophilic pathogenic vibrios *viz.* *V. alginolyticus* and *V. metschnikovii*. (Varma *et al.*, 2003). Due to the halophilic nature and marine origin of *Vibrio* spp. raw seafood is naturally contaminated and is the main food responsible for infection (Desmarchelier, 2003). At present not much information is available regarding the incidence and load of this group of organisms in fish and fish products. The aim of this investigation was to study the incidence and load of halophilic pathogenic vibrios in fish and fish products meant for export and for domestic consumption.

Materials and Methods

One hundred and thirty samples *viz.*, 54 iced, 52 block frozen and 24 individually quick frozen (IQF) samples of fish and fish products were collected from landing centres, markets and fish processing factories situated in and around Cochin meant for export and domestic consumption (Table 1). The samples were examined for the incidence of halophilic pathogenic vibrios. Three Tube Most Probable Number (MPN) technique as described in the Bacteriological Analytical Manual (Anon, 1995) was employed for the enumeration of halophilic pathogenic vibrios. Isolates were identified up to the species level as per the key proposed by Alsina & Blanch (1994).

Results and Discussion

The incidence of halophilic pathogenic vibrios in different fish and fish products are given in Table 1. Halophilic pathogenic vibrios were present in 72% of iced samples, 40.4% of block frozen samples and 29.2% of IQF samples. On an average 51.5% of the samples was found to be positive for halophilic pathogenic vibrios. Iced samples had the maximum load followed by block

frozen and IQF samples. Percentage incidence and load of various species of halophilic pathogenic vibrios in different samples of fish and fish products are given in Table 2-4.

Halophilic pathogenic *Vibrio* spp. were isolated from 72 % of iced samples (Table 1). Among iced samples, the incidence of halophilic pathogenic vibrios were found to be maximum in squid (100%) followed by shrimp (78.9%) and minimum in cuttle fish (42.1%). Incidence of *V. alginolyticus* was high in iced shrimp at 50.5% (load varied from <1 to 360 MPN g⁻¹) followed by *V. vulnificus* at 36.8% (<1 to 530 MPN g⁻¹). The load of *V. parahaemolyticus* and *V. cincinnatiensis* was at 21.0%, (<1 to 7.3 and <1 to 240 MPN g⁻¹), *V. metschnikovii* at 10.5% (< 1 to 30 MPN g⁻¹) and *V. damsela* at 5.3% (<1 to 3.6 MPN g⁻¹). Other species of halophilic pathogenic vibrios were found to be absent. In iced squid samples, the incidence of *V. cincinnatiensis* and *V. vulnificus*

Table 1. Incidence of halophilic pathogenic vibrios in different fish and fish products

Fish products	No. of sample	% incidence of vibrios
Iced		
Shrimp	19	78.9
Squid	13	100.0
Cuttle Fish	19	42.1
Others	3	100.0
Total	54	72.0
Block frozen		
Shrimp	24	45.8
Squid	15	33.3
Cuttle Fish	11	27.3
Others	2	100.0
Total	52	40.4
IQF		
Shrimp	3	33.3
Squid	10	40.0
Cuttle Fish	8	ND
Others	3	66.7
Total	24	29.2

Table 2. Incidence (%) and load (MPN g⁻¹) of various species of halophilic pathogenic vibrios in iced fish products

Vibrios	Load (MPN g ⁻¹) of halophilic pathogenic vibrios in fish products			
	Shrimp	Squid	Cuttlefish	Others
<i>Vibrio alginolyticus</i>	<1-360(50.5)*	<1-150(38.5)	<1-200(10.5)	ND
<i>Vibrio cincinnatiensis</i>	<1-240(21.0)	<1-73(53.8)	<1-930(31.6)	<1-53(33.3)
<i>Vibrio damsela</i>	<1-3.6(5.3)	ND	ND	ND
<i>Vibrio fluvialis</i>	ND	ND	<1(5.3)	ND
<i>Vibrio furnissii</i>	ND	<1-30	ND	ND
<i>Vibrio metschnikovii</i>	<1-30(10.5)	ND	ND	<1(33.3)
<i>Vibrio parahaemolyticus</i>	<1-7.3(21.0)	<1(7.7)	ND	ND
<i>Vibrio vulnificus</i>	<1-530(36.8)	<1-61(53.8)	<1-91(10.5)	ND

* Figures in parenthesis denote % incidence

was found to be high viz. 53.8% (<1 to 73 MPN g⁻¹ and <1 to 61 MPN g⁻¹), followed by *V. alginolyticus* 38.5% (<1 to 150 MPN g⁻¹), *V. furnissii* 15.4% (<1 to 30 MPN g⁻¹) and *V. parahaemolyticus* 7.7% (<1 MPN g⁻¹). Other species of halophilic pathogenic vibrios were found to be absent (Table 2). Incidence of *V. cincinnatiensis* was found to be high in iced cuttlefish viz. 31.6% (<1 to 930 MPN g⁻¹) followed by *V. alginolyticus* and *V. vulnificus* viz. 10.5% (<1 to 200 MPN g⁻¹ and <1 to 91 MPN g⁻¹). *V. fluvialis* was isolated from one sample and its load was <1 MPN g⁻¹. Other species of halophilic pathogenic vibrios were found to be absent.

In block frozen fish products (Table 1), the incidence was high in shrimp (45.8%) followed by squid (33.3%) and cuttle fish (27.3%). Incidence of *V. cincinnatiensis* was found to be high in block frozen shrimp viz. 41.7% (<1 to 9.3 MPN g⁻¹) followed by *V. alginolyticus* 29.2% (<1 to 9.1 MPN g⁻¹), *V. vulnificus* 20.8% (<1 to 11 MPN g⁻¹) and *V. parahaemolyticus* 12.5% (<1 to 6 MPN g⁻¹). Other species were not isolated from the samples (Table 3). In block frozen squid, incidence of *V. alginolyticus* and *V. cincinnatiensis* was found to be high viz. 20.0% (<1 to 20 MPN g⁻¹) followed by *V. furnissii* and *V. metschnikovii* viz., 6.7% (<1). Other species of halophilic pathogenic vibrios were found to be absent. In block frozen

cuttle fish incidence of *V. cincinnatiensis* was found to be high compared to all other pathogenic species viz. 27.2% (<1 MPN g⁻¹) followed by *V. alginolyticus* 9.1% (<1 to 4.3 MPN g⁻¹). Other species of halophilic pathogenic vibrios were found to be absent.

Halophilic pathogenic vibrios were isolated from 40.0% of IQF squid and 33.3% of IQF shrimp samples (Table 1). *V. alginolyticus*, *V. cincinnatiensis* and *V. metschnikovii* were isolated from 33.3% of IQF shrimp samples (<1 to 9.1 MPN g⁻¹). Incidence of *V. alginolyticus* was also found to be high in IQF squid samples viz., 30% (<1 to 1.5 MPN g⁻¹) followed by *V. cincinnatiensis* 20% (<1 to 2 MPN g⁻¹), *V. metschnikovii* and *V. parahaemolyticus* 10% (<1 to MPN g⁻¹). All samples of IQF cuttle fish were found to be free from all species of halophilic pathogenic vibrios (Table 4).

The incidence of *V. cincinnatiensis* was found to be high among halophilic pathogenic *Vibrio* spp. viz., 30.0% (<1 to 930 MPN g⁻¹) in fish and fish products meant for export and domestic consumption followed by *V. alginolyticus*, 25.4% (<1 to 360 MPN g⁻¹), *V. vulnificus*, 16.9% (<1 to 530 MPN g⁻¹), *V. parahaemolyticus*, 8.5% (<1 to 9.1 MPN g⁻¹), *V. metschnikovii*, 4.6% (<1 to 30 MPN g⁻¹), *V. furnissii*, 2.3% (<1 to 30 MPN g⁻¹), *V. damsela* and *V. fluvialis* 0.8% (<1 to 3.6 MPN g⁻¹ and

Table 3. Incidence (%) and load (MPN g⁻¹) of various species of halophilic pathogenic vibrios in block frozen fish products

Vibrios	Load (MPN g ⁻¹) of halophilic pathogenic vibrios			
	Shrimp	Squid	Cuttlefish	others
<i>Vibrio alginolyticus</i>	<1-9.1(29.2)*	<1-9.4(20.0)	<1-4.3(9.1)	<1
<i>Vibrio cincinnatiensis</i>	<1-9.3(41.7)	<1-2.3(20.0)	<1(27.3)	<1
<i>Vibrio damsela</i>	ND	ND	ND	ND
<i>Vibrio fluvialis</i>	ND	ND	ND	ND
<i>Vibrio furnissii</i>	ND	<1(6.7)	ND	ND
<i>Vibrio metschnikovii</i>	ND	<1(6.7)	ND	ND
<i>Vibrio parahaemolyticus</i>	<1-6(12.5)	ND	ND	<1-9.1(100.0)
<i>Vibrio vulnificus</i>	<1-11(20.8)	ND	ND	<1-11(50.0)

* Figures in parenthesis denote % incidence

Table 4. Incidence (%) and load (MPN g⁻¹) of various species of halophilic pathogenic vibrios in IQF fish products

Vibrios	Load (MPN g ⁻¹) of halophilic pathogenic vibrios			
	Shrimp	Squid	Cuttlefish	others
<i>Vibrio alginolyticus</i>	<1-3.6(33.3)*	<1-1.5(30.0)	ND	<1(100.0)
<i>Vibrio cincinnatiensis</i>	<1-9.1(33.3)	<1-2(20.0)	ND	<1-1.5(100.0)
<i>Vibrio damsela</i>	ND	ND	ND	ND
<i>Vibrio fluvialis</i>	ND	ND	ND	ND
<i>Vibrio furnissii</i>	ND	<1(6.7)	ND	ND
<i>Vibrio metschnikovii</i>	<1-3.6(33.3)	<1(10.0)	ND	ND
<i>Vibrio parahaemolyticus</i>	ND	<1(10.0)	ND	ND
<i>Vibrio vulnificus</i>	ND	ND	ND	ND

* Figures in parenthesis denote % incidence

<1). Out of ten halophilic pathogenic *Vibrio* spp., eight were isolated in this study from fish and fish products.

The study showed that halophilic pathogenic vibrios were present in 51.5% of samples consisting of iced, block frozen and IQF fish and fish products meant for export and domestic consumption. Load of *V. parahaemolyticus* in all the samples was found to be less than 10 MPN g⁻¹ (Table 2-4). The standard for *V. parahaemolyticus* in sea food is 10² g⁻¹ which is the only ICMSF standard presently available for halophilic pathogenic vibrios (ICMSF, 1974). Sanjeev &

Stephen (1993) observed that the concentration of *V. Parahaemolyticus* in 15% of fresh finfish and 32.6% of fresh shellfish collected from Cochin exceeded the limit set by ICMSF. None of the 56 prawn samples meant for export had *V. parahaemolyticus* load of more than 100 g⁻¹ (Karunasagar *et al.*, 1984).

As the halophilic pathogenic vibrios are autoctonous to marine and brackish waters it is almost impossible to eliminate these organisms from seafoods (Sunen *et al.*, 1995). As evident from the previous (Sanjeev & Stephen, 1993) and the present study, processing, chilling and freezing can reduce

the incidence of halophilic pathogenic vibrios in fish and fish products. If sea foods are heated at 100°C shortly before consumption, the hazard due to halophilic pathogenic vibrios could be avoided.

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