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Incidence of Listeria in fish and shellfish

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Listeria monocytogenes has been recognized as an important food-borne pathogen since 1981 and seafoods have been implicated in several sporadic cases of Listeriosis. In the present study fresh (57), iced (49) and frozen (84) fish and shellfish from cultured ponds and commercial outlets were screened for Listeriae. Among the samples of fresh cultured fish, 1.8% contained *Listeria innocua*. The iced *Penaeus indicus* (PUD) and *Penaeus monodon* contained 20% *Listeria* spp., comprising *L. innocua* and *L. ivanovii*. *Listeria* spp. were detected in 14.28% of the frozen fish samples also. *Listeria monocytogenes* could not be detected in any of the samples.

Key words : *Listeria* spp, Fish, Shellfish, marine, cultured, Andhrapradesh, cold enrichment

The isolation of *Listeria monocytogenes* from frozen cooked crab meat from a Mexican supplier by a commercial laboratory in USA in 1987 led to enhanced concern about the potential danger of *Listeria* contaminated seafood to public health (Anon, 1987). The first case of Listeriosis linked to consumption of fish and seafood was reported in 1989 (Facinelli *et al*, 1989). Seafood have also been implicated in several sporadic cases of Listeriosis and USFDA requires zero tolerance of *L. monocytogenes* in ready to-eat foods including seafoods (Frediksson, 1991; Fuchs & Reilly, 1992; Baker *et al*, 1993).

The psychrotrophic nature of *L monocytogenes* allows survival or even multiplication of this potential pathogen during refrigeration or temperature-abuse conditions (Jinneman *et al*, 1999). Studies carried out on peelers and processors of seafood processing plants in Mangalore, India, revealed that nearly 4% of the seafood handlers were found to carry *L. monocytogenes* (Jeyasekaran *et al*, 2002a). In the same study floors, drains, tables, containers, and equipments of the seafood processing plants were found to harbour *Listeria* spp. Investigations on prevalence of *Listeria* spp. in processed fishery products namely, frozen shrimps, squids and fish from the seafood processing plants in Mangalore area have revealed that 11.1% samples were found to contain *Listeria* spp. (Jeyasekaran *et al*, 2002b). These studies indicated that the process of chilling and freezing did not completely eliminate *L. monocytogenes* in shrimps (Jeyasekaran *et al*, 2002c). In view of the potential pathogenic nature and public health significance, the present study was undertaken to screen fish and shellfish for Listeria spp. from marine and cultured sources of Andhra Pradesh region.

Materials and Methods

Fish and shellfish samples were collected from commercial outlets and culture ponds in sterile polythene bags and were brought to the laboratory immediately. In case of frozen shellfish, the sample was drawn from all four sides and from centre of the block with a sterile poker in aseptic conditions and after blending, 30 g sample was weighed out aseptically for further analyses.

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Two methods were employed simultaneouly for screening of fish and shellfish for Listeriae. In the first procedure of USDA method as outlined by Fuchs, (1991), 30 g sample was taken into 270 ml of pre-enrichment broth (tryticase soy broth with 0.6% yeast extract). Sample was triturated with small amounts of TSB employing sterile pestle and mortar in aseptic conditions and in the end whole of TSB was mixed. It was incubated at 30°C for 24h. From this, 10ml was taken and mixed in 90 ml of UVM1 and it was incubated at 30°C for 24h. After the incubation, 0.1ml of UVM1 was added to 10ml of UVM II and it was incubated further at 30°C for 24h. The blackened tubes were streaked on MOX (Modified Oxford Medium) and the plates were incubated at 35°C for 24 & 48 h. Colonies surrounded by black halos were picked for further tests.

In the second method, Cold Enrichment procedure was employed for screening the fish and shellfish samples for *Listeriae*. 30 g sample was weighed aseptically and homogenized with 270 ml sterile enrichment broth and was incubated at 4°C. Tryptose phosphate broth (Hi-media, India)) was employed as enrichment broth. At weekly intervals inoculum was taken with sterile loop for subculture on Modified McBrides Agar (MMA) plates. The MMA plates were incubated at 35°C for 48h. Plates were examined using Henry's illumination technique. The composition of MMA was Phenylethanol agar (Difco): 35.5g; Glycine anhydride (Sigma, USA) 10g; Lithium chloride (Sigma, USA) Distilled water 1 l, pH was adjusted to 7.3. The ingredients were heated, dissolved, dispensed and autoclaved at 121°C for 15 min. Prepared bottles (100ml) each were stored at 4°C. After melting the agar, filter sterilized cyclohexamide 20mg/100ml was added. The plates were poured thin and were stored at 4°C and were used within week's time whenever necessary.

Suspected colonies were picked up and were purified on Tryticase Soy Agar (TSA) with 0.6% yeast extract (YE) plates. The isolates were further tested for morphological and biochemical characters by standard methods (Jones & Seeliger, 1992).

L. monocytogenes NCTC (11994), *Staphylococcus aureus* (NCTC 1803) and *Rhodococcus equi* NCTC (1621) were the test cultures used in this study.

Results and Discussion

Incidence of Listeria in fresh fish is shown in Table 1. Out of 57 samples screened from culture ponds and local outlets, only *Labeo rohita* harboured *Listeria spp* and it was identified as *L.innocua*. *L. innocua* is not considered to cause disease in humans (Rocourt & Seeliger, 1985) and has been reported in 33% freshly caught marine and brackish fish samples (Fuchs & Surendran, 1989).

Occurrence of Listeria in iced fish and shellfish in the present study are shown in Table 2. *Listeria monocytogenes* was not detected in iced fish, *Penaeus indicus* (Indian

Table 1. Incidence of Listeria in fresh fish (n= 57)

No. of samples screened	Description of the sample	Source	Samples Positive for *Listeria* spp.
10	*Hypopthalmychthys molitrix*	Local outlet	Negative
10	*Cirrhinus mrigala*	Culture Ponds	Negative
10	*Labeo rohita*	Culture Ponds	Positive (1) *L. innocua*
5	*Johnius dussumeiri*	Local outlet	Negative
17	*Mugil cephalus*	Local outlet	Negative
5	*Megalops cundinga*	Local outlet	Negative

Table 2. Incidence of Listeria in iced fish and shellfish (n=49)

No.	Description of the sample	Source	Samples Positive for *Listeria* spp.	Listeria spp isolated / No. samples
4	*Cirrhinus mrigala* (iced for 5 days)	Culture Ponds	Nil	
3	*Cirrhinus mrigala* (kept on ice for 13 days)	Culture Ponds	Nil	
16	*Penaeus indicus* (PUD)	Local outlets	3	2 *Listeria innocua* (2) *Listeria ivanovii* (1)
9	*Penaeus monodon*	Local outlets	2	*L.innocua* (1) *L.ivanovii* (1)
11	*Penaeus indicus* (P.D)	Local outlets	Nil	
6	*Parapenaeopsis stylifera* (8 days in ice)	Local outlets	Nil	

white prawn) (PD) or in deep-sea shrimp. Other *Listeria* spp. were detected in 10.2% of the iced fish and shellfish samples screened. Among the *Penaeus indicus* (PUD) and *Penaeus monodon*, 20% were positive for *Listeria* spp., and the predominant species were *Listeria innocua* followed by *L. ivanovii*.

The occurrence of *Listeria* in frozen fish and shellfish samples is depicted in Table 3. Out of 84 samples of frozen fish and shellfish screened 14.28% were positive for *Listeria* spp. *Listeriae* were not detected in any of the deep-sea prawns and fish brought for analysis. Both PUD and PD *P. indicus* harboured *Listeria* spp., at 18.75 and 16.66%, respectively. Interestingly *L innocua* is dominant flora in PUD prawns while PD prawns harboured mainly *L. ivanovii*. In this study occurrence of *L. monocytogenes* was not seen in any of the samples screened. Earlier reports also did not show occurrence of *L. monocytoenes* in seafoods. However, *L. innocua* and *L. grayi* were detected (Fuchs & Surendran 1989; Kamat & Nair 1994).

In the present study the incidence of *Listeria* spp is more in shrimps than in fishes. This is in agreement with the report of Jeyasekaran *et al* (2002b) in which the

Table 3. Incidence of Listeria in frozen fish and shellfish (n=84)

No. of samples screened	Sample	Source	Samples Positive for *Listeria* spp.	Listeria spp/ No. positive
3	*Nemipterus japonicus*	Market	Nil	–
3	*Upenus vittatus*	Market	Nil	–
6	*Parapenaeopsis stylifera*	Deep sea	Nil	–
16	*P. indicus* (PUD)	Local outlets	3	*L. innocua* (2) *L. ivanovii* (1)
8	*P. monodon* (Headless)	Local outlets	1	Positive (1) *L. innocua*
18	*P. indicus* (PD)	Local outlets	3	Positive (3) *L. innocua* (1) *L. ivanovii* (2)
10	*P. monodon* (PUD)	Local outlets	2	*L. ivanovii* (2)
20	*Metapenaeus dobsoni*	Local outlets	3	*L. innocua* (1) *L. ivanovii* (2)

Table 4. Comparison of detection of Listeria by cold enrichment and USDA methods

Sample	No samples positive for Listeria		No. of isolates positive for Listeria by	
	Cold enrichment	USDA	Cold enrichment	USDA
			No. positive / total isolates	No. positive / total isolates
Fresh fish	1	1	4/4	4/4
Fresh shrimp	None	None	NA	NA
Iced fish	Not detected	Not detected	Not detected	Not detected
Iced shrimp	5	5	25/25	22/25
Frozen fish				
Frozen shrimp	12	11	77/77	61/72
Total	18	17	106	87

occurrence of *Listeria spp*. in shrimps was very high (14.3%), when compared to its incidence in finfishes (4.3%). The positive isolates were identified as *L. monocytogenes* and *L. innocua* (Jeyasekaran *et al*, 2002b), whereas, in the present study the distribution in occurrence of *L. ivanovii* and *L innocua* was equal. The occurrence of *Listeria innocua* in sea food samples is also reported by Weagent *et al*, (1988), Fuchs & Sirvas, (1991) and Jeyasekaran *et al*, (2002b).

A comparison of detection of *Listeria* from different fish and shellfish samples by cold enrichment method and Listeria isolation method is shown in Table 4. A perusal of data in the Table 4 reveals that wherever *Listeria* presence is detected by cold enrichment method in the fresh, iced and frozen fish and shell fish samples (!8), all the suspected Listeria isolates were confirmed as *Listeria* spp while in the USDA Listeria isolation method confirmation of the suspected isolates were slightly lower (17/18) in comparison with cold enrichment method. Hayes *et al* (1991) made similar observations in the study on comparison of cold enrichment methods and USDA-FSIS method. Biester & Schwarte (1939) reported for the first time detection of Listeria by cold enrichment method. Fatty acid (anteiso-$C_{15:0}$) is reported to play an important role in adaptation of *Listeria* to cold temperatures, with mutants deficient in this fatty acid being shown to be cold sensitive (Annous *et al*, 1997).

During the process of isolation, some colonies were seen to have morphological characters similar to Listeriae. However, the major hindrance was subculture on TSA plates. In this study 14 of the 120 suspected Listeria isolates belonged to this category. Since these isolates were from iced or processed samples, there is a possibility for viable but non-culturable (VBNC) state. Besnard *et al*, (2000) examined factors inducing *L. monocytogenes* into the VBNC state and concluded that inoculum size, temperature (4°C and 20°C), NaCl concentration (0-7%), pH (5 and 6), and presence of sunlight were contributory factors for development of VBNC state. In the present study the samples were exposed to all the conditions mentioned at different stages of post harvest handling. The presence of VBNC cells of this pathogen if present, could pose a major public health hazard since the cells cannot be detected by routine culture methods (Oliver, 2005). The study of Cappelier *et al*, (2005) suggested that *Listeria monocytogenes* might remain in the aquatic environment for prolonged period in the VBNC state; but did not exhibit any pathogenicity in the conditions tested and stressed the role of VBNC cells in environmental transmission and virulence of *L. monocytogenes*.

This study revealed that out of 190 fresh, iced and frozen shellfish samples screened 9.5% of them harboured *Listeria spp*

with equal distributon of *L. innocua* and *L. ivanovii*. None of the samples screened were positive for *Listeria monocytogenes*. Cold enrichment facilitated higher number of confirmed isolates for *Listeria spp* than the USDA method.

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