Fishery Technology
2010, Vol. 47(2) pp: 195 - 200

Research Note

Incidence of Food-borne Pathogens in Freshwater Fish from Domestic Markets of Mumbai

A. S. Kakatkar, R. K. Gautam, V. Nagar, M. Karani and J. R. Bandekar[1]
Food Microbiology and Seafood Technology Section, Food Technology Division, Bhabha Atomic Research Centre, Mumbai - 400 085, India

Food-borne diseases are associated with fish and fishery products. Biotoxins, histamines and viruses cause large proportion of these outbreaks. Fishery products are also recognized as carrier of food-borne bacterial pathogens like *Salmonella* spp., *Vibrio* spp., *E. coli*, *S. aureus*, and *Listeria* spp. (Venugopal *et al.,* 1999, 2002). The pathogenic organisms are present on the fish itself or in the aquatic environment around the fish; however, the presence of pathogenic bacteria in and around fish is low (Huss, 1997). Studies on seafood meant for export from India have shown that a large percentage of these products are contaminated with *Salmonella* (Varma *et al.,* 1985; Kamat *et al.,* 2003; Kumar *et al.,* 2003; Bandekar *et al.,* 2004).

Aquaculture is one of the fastest growing industry in the world. The annual fresh water fish production in India constitutes 93.1% of the total 2.83 million metric tonnes of the aquaculture resources and is mainly consumed in the domestic sector (Bhat, 2008). *Salmonella spp.* and *Listeria spp.* have been reported in aquacultured fish (Nedoluha & Westhoff, 1997; Kamat *et al.,* 2003; Bandekar *et al.,* 2004; Saroj *et al.,* 2008a). Although, there are some reports on the bacteriological quality and incidences of various pathogens in fresh water fish and cultured shrimp (Ahmed, 1995; Bhaskar, 1998; Sapana, 2001), detailed study on microbiological quality of different species are lacking. The objective of this study was to generate information on the microbiological quality and incidence of bacteria of public health significance in six species of freshwater fish marketed in Mumbai during the period from May 2007–March 2008. The pathogens covered in this study were *Salmonella* spp., *Vibrio* spp, coagulase positive *S. aureus, Y. enterocolitica* and *L. monocytogenes*.

Forty two samples of six different species of freshwater fish, Butter catfish (*Ompok bimaculatus)*, Tengan (A*ristichithys nobilis*), Hilsa (*Tenulosa ilisha*), Mangur (*Clarias batrachus*), Catla (*Catla catla*) and Rohu (*Labeo rohita*) were procured from local fish market, brought to the laboratory in ice under sterile conditions and microbiological analysis was done within one hour.

Microbiological media used in this study were from HiMedia Laboratories, Mumbai, India. Rabbit plasma was from Becton Dickinson (Sparks, Md. USA). The chemicals required for PCR amplification were from Banglore Genei, India. The primers used in this study were procured from BRIT, Mumbai, India.

Microbiological analysis was performed as per standard methods adopted from online *Bacteriological Analytical Manual,* U.S.

[1] Corresponding author; e-mail: jrb@barc.gov.in

Food and Drug Administration, for the detection, enumeration, and identification of individual organisms (BAM, 2006). *Salmonella* serovar Typhimurium MTCC 98 (Microbial Type Culture Collection, Chandigarh, India), *Listeria monocytogenes* NCAIM-B-01442 (an avirulent strain, supplied by Dr. Cs. Mohacsi-Farkas, Szent Istvan University, Budapest, Hungary), *Escherichia coli* ATCC 35218, coagulase positive *S. aureus* isolated from poultry and *Y. enterocolitica* MTCC 859 were used as standards for biochemical tests.

Aerobic plate count (APC) was performed by homogenizing 25 g sample in 225 ml of sterile physiological saline. After appropriate serial dilutions, the samples were pour-plated on plate count agar. The colonies were counted after 48 h of incubation at room temperature (30°C).

For detection of coliforms, appropriate dilutions from saline were pour plated on violet red bile agar (VRBA), and after the medium was solidified, it was overlaid with VRBA. Typical dark red colonies were counted after 24 h of incubation at 37°C and the isolates were subjected to indole, methyl red, Voges-Proskauer and citrate tests (IMViC test) to confirm *E. coli.* The spread plate technique was used to determine the Staphylococci counts on Baird-Parker agar (BPA). Dilutions made for APC were plated on BPA. After 24 to 48 h incubation at 37°C, the characteristic black colonies with a peripheral clearance zone were selected. Coagulase activity was confirmed by checking the clot formation in brain heart infusion broth with 2% rabbit plasma. For the isolation of *Salmonella*, *Vibrio*, *Listeria* and *Yersinia*, 25 g of samples were pre-enriched in 225 ml of the respective pre-enrichment broth, followed by selective enrichment if required and then streaked on selective agar plates as per BAM (2006). The selective agars used for various organisms were bismuth sulfite agar, xylose lysine deoxycholate agar, and Hektoen enteric agar for *Salmonella*, thiosulphate citrate bile sucrose agar for *Vibrio*, *Listeria* identification agar for *Listeria*, MacConkey agar for *Yersinia*. All the plates were incubated at 37° C for 24 h except for MacConkey's agar plates which were incubated for 18 to 24 h at 30°C. Typical colonies from respective selective media were picked and identified by biochemical tests. Isolates for *Salmonella* confirmed by biochemical tests were checked for the presence of *inv*A gene by PCR method (Shashidhar *et al*., 2005). These isolates were serotyped at National Salmonella and Escherichia Centre, Kasauli, India. Species level identification of presumptive positive *Vibrio* isolates was carried out as per BAM (2006). Isolates confirmed biochemically as *V. cholerae* were checked for the presence of *ctx* gene as described by online BAM (2006).

The APC of all the samples was in the range of 10^7 to 10^8 cfu/g. The coliform count and S*taphylococcus* count for all the samples were in the range of 10^5 to 10^6 cfu/g (Fig. 1). The fresh water fish sold in the local market in Mumbai are brought from Andhra Pradesh and Kolkota in iced condition with a transportation time of one to two days. The high APC as well as coliform and *Staphylococcus* count in the fish samples may be due to temperature abuse during transportation and/or handling and processing.

Nineteen percentage of samples were found to be positive for *Salmonella* by biochemical tests. All the *Salmonella* isolates were positive for *inv*A gene by PCR. The *inv*A gene has been shown to be specific for *Salmonella* and it has been used for the rapid detection of *Salmonella* (Chiu & Ou, 1996). All the fish species studied, except Tengan, were contaminated with *Salmonella* indicating wide prevalence of this pathogen. The *Salmonella* isolates were serotyped as *S.* Weltevreden, *S.* Oslo, *S.* Typhimurum and *S.* Derby. *S.* Oslo was present in all the fish

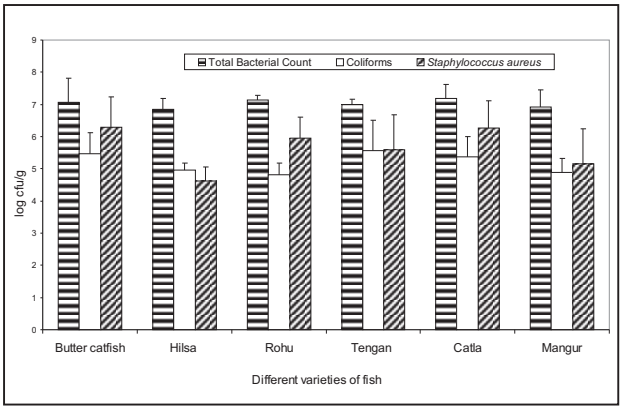

Fig 1. Microbial load in different varieties of fish

samples that were found positive for *Salmonella*. *S.* Weltevreden was detected in Butter catfish and Mangur while *S.* Typhimurium was detected in Catla and Rohu. *S.* Derby was detected only in Catla sample (Table 1). Presence of *Salmonella* in seafoods has been reported from India, Thailand, Japan, Vietnam, Sri Lanka and US (Heinitz *et al*., 2000). In India, *Salmonella* has been reported in fish, squid, crustaceans, cuttle fish and scampi (Hatha & Lakshmanaperumalsamy, 1997; Kamat *et al*., 2003; Bandekar *et al*., 2004; Saroj *et al*., 2008a). Presence of *S*. Typhimurium and *S.* Weltevreden has also been reported from fish and crustaceans (Shabrinath *et al*., 2007). Bandekar *et al*. (2004) reported presence of *S.* Worthington and *S.* Typhimurium in scampi. *S*. Typhimurium was the most common serovar in seafoods reported world wide including India (Saroj *et al*., 2008b). Recently, *S.* Weltevreden has been reported to be the most frequent serovar in imported seafood samples as analyzed by USFDA (Heinitz *et al*., 2000). Ponce *et al*. (2008) reported S. Weltevreden as the most common serovar from seafood samples in South East Asia. It is also common in aquacultured shrimp farms. The sources of contamination are contaminated feeds, animal faeces and manure added to the shrimp farms (Bhaskar *et al*., 1998). *S*. Oslo was reported from seafoods in India for the first time by Singh *et al*. (1997). *S*. Derby has been isolated from slaughter pigs and other food and also from cases of human salmonellosis (Michael *et al*., 2006).

Escherichia coli was present in 14.2% of Catla and Mangur, 28.5% of Tengan and 42.8% of Hilsa samples. Butter catfish and Rohu were found to be free from *E. coli*. The presence of *E. coli* indicates faecal contamination, as *E. coli* constitute the normal flora of human and animal intestine. The presence of coagulase positive *S. aureus* was from 14.2% of Tengan and 28.5% of Hilsa samples. 14.2% of Butter catfish and Tengan were

Table 1. Incidence of pathogens in fish

Name of fish	No. of positive samples / Total samples				
	*Salmonella** spp.	*Vibrio*** spp.	*Listeria* spp.	Coagulase positive *Staphylococcus aureus*	*Yersinia enterocolitica*
Butter catfish	1/7	1/7	0/7	0/7	0/7
Catla	2/7	0/7	0/7	0/7	0/7
Rohu	2/7	0/7	0/7	0/7	0/7
Mangur	2/7	0/7	0/7	0/7	0/7
Tengan	0/7	1/7	0/7	1/7	0/7
Hilsa	1/7	0/7	0/7	2/7	0/7

**Salmonella* serotypes *S.* Typhimurium, *S.* Weltevreden, *S.* Oslo and *S.* Derby were detected
***V. cholerae* and *V. mimicus* were detected

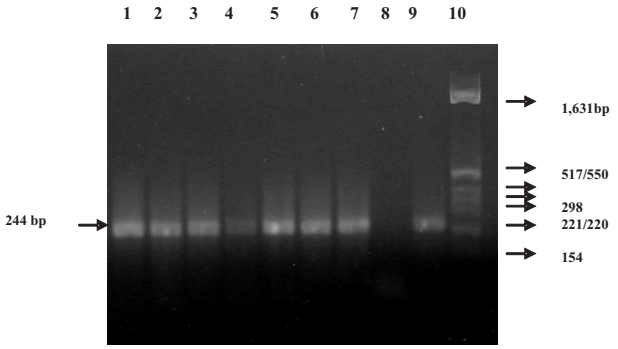

Fig. 2. Agarose gel electrophoresis of different Salmonella isolates showing *inv*A gene. lane 1, 2, 3 represent S. Weltevereden, 4 S. Derby, 5 & 6 S. Oslo, 7 S. Typhimurium, 8 Negative control, 9 S. Typhimurium MTCC 98 and 10 Marker pBR 322

positive for *Vibrio* spp. Butter catfish sample was positive for *V. cholerae*, while Tengan fish was positive for *V. mimicus*. However, *ctx* gene was absent in *V. cholerae* isolate indicating that the isolate was non-toxigenic. Huss (1997) also reported presence of non-toxigenic non-O1 *V. cholerae* in shrimps exported to Denmark. *V. cholerae* has been reported in seafoods and coastal environments of southwest India (Saravanan *et al.,* 2007). *V. mimicus* is also the normal flora of aquatic environment (Bhaskar *et al.,* 1998). *Listeria* spp. was not present in any of the samples. Previous studies from southern India and western India have also reported absence of *L. monocytogenes* in fish (Kamat & Nair, 1994; Antony *et al.,* 2002; Kamat *et al*., 2003; Bandekar *et al*., 2004; Das *et al*., 2008;). It is observed that prevalence of *L. monocytogenes* in temperate regions (4-12%) is more compared to tropical regions (0-2%) (Davies *et al*., 2001). Out of 167 presumptive positive *Yersinia* isolates, 6 showed typical biochemical characteristics. However, none of them were *Y. enterocolitica* based on biochemical tests. Khare *et al*. (1996) reported presence of *Yersinia* spp. in fish.

The present study indicates that the freshwater fish sold in Mumbai are of poor quality. Presence of coagulase positive *S. aureus* indicates unhygienic handling. Recovery of *Salmonella* and high coliform count warrant for strict adherence to Good Hygienic Practices in retail markets.

The authors are thankful to Dr. Cs. Mohacsi-Farkas, Szent Istvan University, Budapest, Hungary for providing avirulent strain of *Listeria monocytogenes* and to the Director, National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, India for serotyping of *Salmonella* isolates. The kind assistance rendered by Mrs Vaishali V. Mahale during the entire work is also acknowledged.

References

Ahmed, N., Karunasagar, I. and Karunasagar, I. (1995) Microbiology of cultured shrimp in India Research contribution presented at the *9th session of the Indo-pacific fishery commission working party on fish technology and marketing*. Food and Agriculture Organization, Fisheries Report **514**, pp 13-22 (Suppl)

Antony, M. M., Jeyasekaran, G., Jeya Shakila, R. and Shanmugam, S. A. (2002) Microbiological quality of raw shrimps processed in seafood processing plants of Tuticorin, Tamil Nadu, India, *Asian Fish. Sci.* **15**, pp 33-41

BAM (2006) Online *Bacteriological Analytical Manual, 8th edn., U. S. Food and Drug Administration,* Arlington, Available http://www.cfscan.fda.gov/ebam/bam-mm.html. Last Accessed on 24. 10. 2008

Bandekar, J. R., Kamat, A. S., Karani, M., Dhokane,V., Shashidhar, R., Kakatkar, A., Ghadge, N., Bhat, A., Venugopal, V. and Warrier, S. B. (2004) Bacteriology quality of farmed freshwater fish and shellfish meant for export, *Fish. Technol.* **41**, pp 57-62

Bhaskar, N., Setty, T. M. R., Mondal, S., Joseph, M. A., Raju, C. V., Raghunath, B. S. and Anantha, C. S. (1998) Prevalence of bacteria of public health significance in the cultured shrimp (*Penaeus monodon*) *Food Microbiol.* **15**, pp 511-519

Bhat, B. V. (2008) Export oriented aquaculture in India: An overview, *Fishing Chimes* **27**, pp 10-11

Chiu, C., and Ou, J. T. (1996) Rapid identification of *Salmonella* serovars in faeces by specific detection of virulence genes invA and spvC by an enrichment broth culture-multiplex PCR combination, *J. Clinical Microbiol*. **34**, pp 2619-2622

Das, S., Surendran, P. K. and Thampuran, N. (2008) Isolation and PCR of *Listeria* spp. from fishes of Cochin area, *Fish. Technol.* **45,** pp 73-78

Davies, A. R., Capell, V., Jehanno, V., Nychas, G. J. E. and Kirby, R. M. (2001) Incidence of food borne pathogens on European fish, *Food Cont.* **12**, pp 67-71

Heinitz, M. L., Ruble, R. D., Wagner, D. E. and Tatini, S. R. (2000) Incidence of *Salmonella* in fish and seafood, *J. Food Prot.* **63**, pp 579-592

Huss, H. (1997) Control of indigenous pathogenic bacteria in seafood, *Food Cont.* **8**, pp 91-98

Kamat, A. S. and Nair, P. M. (1994) Incidence of *Listeria* species in Indian seafoods and meat, *J. Food Safety* **14**, pp 117-130

Kamat, A.S., Panda, S.K., Karani, M., Pingulkar, K., Ghadge, N., Jadhav, S. S., Venugopal, V., Warrier, S. B., Bandekar, J. R. and Bongirwar, D. R. (2003) Microbiological quality of some seafood products exported from Mumbai, India, *Asian J. Microbiol. Biotechnol. Environ. Sci.* **5**, pp 2977-2980

Khare, S. S., Kamat, A. S., Doctor, T. R. and Nair, P. M. (1996) Incidence of *Yersinia enterocolitica* and related species in some fish meat and meat products in India, *J. Sci. Food Agri.* **72**, pp 187-195

Kumar, H. S., Suni, R., Venugopal, M. N., Karunasagar, I. and Karunasagar, I. (2003) Detection of *Salmonella* spp. in tropical seafood by polymerase chain reaction, *Int. J. Food Microbiol.* **88**, pp 91-95

Michael, G. B., Cardoso, M., Rabsch, W. and Schwarz, S. (2006) Phenotypic and Genotypic differentiation of porcine *Salmonella enterica* subsp. *enterica* serovar Derby isolates, *Vet. Microbiol.* **118**, pp 312-318

Mohamed Hatha, A. A. and Lakshamanaperumalsamy, P. (1997) Prevalance of *Salmonella* in fish and crustaceans from markets in Coimbatore, South India, *Food Microbiol.* **14**, pp 111-116

Nedoluha, P. C. and Westhoff, D. (1997) Microbiology of striped bass grown in three aquaculture systems, *Food Microbiol.* **14**, pp 255-264

Ponce, E., Khan, A. A., Cheng, C. M., West, C. S. and Cerniglia, C. E. (2008) Prevalance and characterization of *Salmonella enterica* serovar *Weltevreden* from imported seafood, *Food Microbiol.* **25**, pp 29-35

Sapna, K. Prasad, B. N., Gita, K., Quasim, A., Basant, K. S. and Singh, J. N. (2001) Microbiological quality of fish, Rohu marketed in Patna and its public health significance, *J. Food Sci. Technol.* **6**, pp 607-608

Saravanan, V., Kumar, H. S., Karunasagar, I. and Karunasagar, I. (2007) Putative virulence genes of *Vibrio cholerae* from seafoods and the coastal environments of South West India, *Int. J. Food Microbiol.* **119**, pp 329-333

Saroj, S. D., Shashidhar, R. and Bandekar, J. R. (2008a) Genetic Diversity of food isolates of *Salmonella enterica* serovar *Typhimurium* in India, *Int. J. Food Sci. Technol.* **14**, pp 151-156

Saroj, S. D., Shashidhar, R., Karani, M. and Bandekar, J. R. (2008b) Distribution of

Salmonella pathogenicity island SPI-8 and SPI-10 among different serotypes of *Salmonella*, *J. Med. Microbiol.* **57**, pp 426-427

Shabrinath, S., SanathKumar, H., Khushiramani, R., Karunasagar, I. and Karunasagar, I. (2007) Detection and characterization of *Salmonella* associated with tropical seafood, *Int. J. Food Microbiol.* **114**, pp 227-233

Shashidhar, R., Jajoo, S., Karani, M., Warrier, S. B. and Bandekar, J. R. (2005) Studies on *Salmonella* isolates from fishery products, *Fish. Technol.* **42**, pp 217-224

Singh, H., Dewan, M. L., Daniel, J. and Koshi, G. (1997) Isolation of *Salmonella abaetetuba* and *Salmonella oslo* for first time in India, *Ind. J. Microbiol.* **17**, pp 85-87

Varma, P. R. G., Mathen, C. and Mathew, A. (1985) Bacteriological quality of frozen seafoods for export with special reference to *Salmonella* In: *Harvest and Postharvest Technology of Fish* (Ravindran, K., Nair, N. U., Perigreen, P. A., Madhavan, P., Pillai, A. G. G. K., Panicker, P. A. and Thomas, M., Eds) pp 483- 484, Society of Fisheries Technologists (India). Cochin

Venugopal, V., Doke, S. N. and Thomas, P. (1999) Radiation processing to improve the quality of fishery products, *Crit. Rev. Food Sci. Nutr.* **39**, pp 341-440

Venugopal, V., Warrier, S. B., Bandekar, J. R. and Bongirwar, D. R. (2002) Radiation processing for control of biohazards in aquaculture, *Fish Farmer Int.* **25**, pp 30-31