ELSEVIER

Food Microbiology 22 (2005) 133–137

FOOD MICROBIOLOGY

www.elsevier.nl/locate/jnlabr/yfmic

Short Communication

Prevalence of *Aeromonas hydrophila* in fish and prawns from the seafood market of Coimbatore, South India

G. Vivekanandhan[a,*], A.A.M. Hatha[b], P. Lakshmanaperumalsamy[c]

[a] *Laboratory of Fish Health Management, Tokyo University of Marine Science and Technology, 4-5-7, Kounan, Minato-ku, Tokyo 108-8477, Japan*
[b] *Department of Biology, School of Pure and Applied Sciences, The University of the South Pacific, P.O. Box 1168, Suva, Fiji*
[c] *Department of Environmental Sciences, Bharathiar University, Coimbatore 641 046, Tamil Nadu, India*

Received 3 September 2003; accepted 26 January 2004

Abstract

Five hundred and thirty six samples of fishes and 278 prawn samples from the major fish market of Coimbatore, South India, were analysed for the prevalence of *Aeromonas hydrophila* over a period of 2 years (June 1997–May 1999). The prevalence level of *A. hydrophila* varied from 17.62% in prawns to 33.58% in fishes. More than 30% of the popular table fishes such as *Sardinella longiceps*, *Rastrelliger kanagurta*, *Mugil cephalus* and *Caranx sexfasciatus* were tested positive for this organism. Among the different species of the prawns analysed, *Penaeus semisulcatus* showed higher incidence (23.52%). Seasonal variation in the prevalence levels of *A. hydrophila* in fish and prawns revealed a higher prevalence during the monsoon season during 1997–98 and 1998–99. Of the different body parts of the fishes analysed for *A. hydrophila*, the intestinal samples showed higher prevalence (38.43%), followed by body surface (32.46%) and gill (29.10%). Considering the psychrotrophic nature and role of *A. hydrophila* as a pathogen of emerging importance, the considerably high levels of this organism in a popular food item such as fish and prawn raises serious concern.

Keywords: *Aeromonas hydrophila*; Fish; Prawn

1. Introduction

Aeromonas spp. represents a group of ubiquitous micro-organisms of aquatic environments such as fresh water, coastal water and sewage (Monfort and Baleux, 1990). These bacteria have a broad host range and have often been isolated from humans with diarrhoea (Ashdown and Koehler, 1993; Janda and Abbott, 1998), though they are recognized as primary pathogens to a wide range of cold blooded animals, in particular to fish (Austin and Adams, 1996). Strains isolated from the environment do not seem to differ from strains isolated from cases of infection with respect to the prevalence of virulence factors (Krovacek et al., 1994). However, it has been reported that certain species are more frequently isolated from patients with diarrhoea as well as from diseased fish than from the environment (Kirov et al., 1994).

A variety of foods have been shown to harbour motile aeromonads including seafoods (Abeyta and Wekell, 1988), raw milk, sausage (Buchanan and Palumbo, 1985) chicken, fresh beef and pork (Okrend et al., 1987), lamb and meat offal (Majeed et al., 1989). Hudson and Delacy (1991) have examined the incidence of motile aeromonads in a variety of uncooked and ready to eat foods.

Coimbatore is a cosmopolitan, land locked city in Tamil Nadu, South India. Fishes and prawns are popular food item and the consumption is steadily increasing owing to their nutritional value and affordability when compared to other foods of animal origin. The demand for fish is met by the fishes transported from neighbouring coastal states such as Kerala and Andhra Pradesh as well as from the distant coastal city of Rameswaram in Tamil Nadu. *Aeromonas* spp. being ubiquitous in the aquatic environment has been reported from wild fish, pond cultured edible and ornamental fish

*Corresponding author. Tel./fax: +81-3-5463-4054.
E-mail addresses: aerovivek@hotmail.com (G. Vivekanandhan), abdulla_m@usp.ac.fj (A.A.M. Hatha), lps@mailcity.com (P. Lakshmanaperumalsamy).

0740-0020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fm.2004.01.015

(Palumbo et al., 1985; Tsai and Chen, 1996). Prevalence of *A. hydrophila* in shrimps has also been reported (Gobat and Jemmi, 1993; Granum et al., 1998). However, most of these studies have been carried out in other developed countries and no systematic study has been carried out in the study area. Though there are few reports available on the prevalence of motile aeromonads in the gastrointestinal tract of farm raised fresh water fishes (Hatha et al., 2000; Hatha, 2002) virtually nothing is available on the prevalence of this organism in marketed fish and prawns. Considering ubiquitous nature of *Aeromonas* spp. in the aquatic environment, the psychrotrophic nature of the organism, the long hours of travel involved in the transportation of the seafood to Coimbatore and finally the increasing role of *A. hydrophila* as human pathogen we decided to take up a systematic study on the prevalence of this organism in the fish and prawn samples collected from major fish market of Coimbatore city.

2. Methods

2.1. Description of the study area

Coimbatore (Lat. 11°N; Long. 71°E) is a landlocked district in Tamil Nadu and the fishes and crustaceans reaching here are caught in the South Indian coasts and transported to Coimbatore. The only entry point is Ukkadam fish market, where fishes from Cochin and Calicut in Kerala, Rameswaram in Tamil Nadu and Mangalore in Karnataka are reaching everyday and distributed to different parts of the district. The daily average inflow is more than 6 tonnes.

2.2. Collection of samples

Fish and prawn samples were collected from the Ukkadam, major market of Coimbatore for a period of 2 years (June 1997–May 1999). Samples were collected at random from a number of vendors in the fish market. Collections were made between 7.00 A.M. and 9 A.M. The fishes and prawns were collected individually in presterilized polyethylene bags and transported to the laboratory in an ice chest. Samples of fish and prawns with visible signs of deterioration, injury or disease were culled. Processing and inoculation of samples for bacteriological analysis were completed within 2–4 h of collection (AOAC, 1975). Aseptic procedures were strictly followed during collection, transportation and analysis. The fishes and prawns were identified using standard reference manuals (Day, 1889; Munro, 1955; Misra, 1959; Tirmizi, 1967; Whitehead, 1972).

2.3. Bacteriological methods

All the specimens were rinsed with sterile water to remove the adhering particles. The body surface of the fish was swabbed with sterile cotton swab. Using an ethanol dipped and flamed forceps, the operculum of the fishes was lifted and the gill surface was swabbed on both the flanks. Using a pair of sterile scissors, an incision was made near the vent of the fish exposing the rectum to facilitate swabbing of faecal matter. Swabs were then transferred to alkaline peptone-water (APW) and incubated at 37°C for 18 h. Whole prawns were dipped into screw cap bottles containing APW so as to transfer the bacterial load into APW. Prawns were removed from the bottles after dipping for 2 min.

After incubation, a loopful of the APW culture was streaked on starch ampicillin agar medium (Himedia, Mumbai, India) and incubated at 37°C for 18–24 h as described by Palumbo et al. (1985). The plates were then flooded with approximately 5 ml of Lugol's iodine solution and amylase positive yellow to honey coloured colonies were isolated. The isolated cultures were then purified by repeated streaking on nutrient agar and maintained in nutrient agar slants. The pure cultures were identified as presumptive *A. hydrophila* using the multitest medium of Kaper et al. (1979). Tubes with alkaline slant and acid butt after 24 h at 37°C were considered as presumptive positive for *A. hydrophila*. The presumptive isolates were confirmed as *A. hydrophila* based on the following reactions: motile, Gram-negative, cytochrome oxidase positive, D-glucose fermentation positive, arginine dihydrolase positive, ornithine decarboxylase negative, ONPG positive, H_2S from cystein, acetoin from glucose, gas from glucose, L-arabinose utilization and fermentation of salicin. We have used a type strain of *A. hydrophila* (MTCC 646), as reference strain to compare the results.

3. Results and discussion

The number of fishes and prawns analysed during each month and the prevalence of *A. hydrophila* during each month is given in Table 1. Out of 536 fishes analysed, 180 (33.58%) fishes were found to be contaminated with *A. hydrophila*. The level of incidence in fish samples was higher when compared to the observations of Tsai and Chen (1996), Fricker and Tompsett (1989) and Hudson and Delacy (1991) from different geographical regions. Higher prevalence in Indian markets is possible due to the poor sanitation and time/temperature abuse of this highly perishable food in the markets. Fishes are left open with little ice and fly infestation is common. However the prevalence levels were much lower than those reported by Abeyta and Wekell (1988) and Gobat and Jemmi (1993) in fresh

Table 1
Prevalence *A. hydrophila* in the marketed fish and prawns during June 1997–May 1999

Month of collection	Prevalence (%)	
	Fish	Prawn
June, 1997	29.16 (24)[a]	0.0 (8)[a]
July	29.62 (27)	55.55 (9)
August	47.82 (23)	0.0 (11)
September	52.84 (17)	33.33 (6)
October	46.42 (28)	0.0 (4)
November	17.24 (29)	33.33 (12)
December	25.00 (20)	0.0 (3)
January, 1998	20.00 (10)	13.63 (22)
February	33.33 (3)	0.0 (6)
March	4.34 (23)	0.0 (6)
April	58.33 (12)	21.73 (23)
May	22.22 (18)	16.66 (12)
June	37.50 (24)	17.64 (17)
July	37.50 (32)	36.36 (11)
August	41.37 (29)	33.33 (9)
September	52.38 (21)	9.09 (11)
October	34.61 (26)	16.66 (12)
November	20.00 (5)	16.66 (24)
December	28.00 (25)	28.57 (7)
January, 1999	30.76 (26)	14.28 (7)
February	33.33 (30)	0.0 (11)
March	45.45 (33)	31.25 (16)
April	25.00 (28)	15.78 (19)
May	26.08 (23)	0.0 (12)
	33.58 (536)	17.62 (278)

[a] Figure in the parenthesis indicates the number of samples analysed.

Table 2
Prevalence of *A. hydrophila* in different fishes analysed during June 1997–May 1999

Name of fish	Prevalence (%)
Ambassis commersoni	0 (1)[a]
Anchoviela commersoni	28.12 (32)
Arius jella	7.14 (14)
Anguilla bicolar	0 (4)
Belone strongilura	0 (4)
Callyodon fasciatus	0 (3)
Caranx sexfasciatus	38.77 (49)
Chaetodon trifasciatus.	66.66 (3)
Cybium commersoni	30 (20)
Epinephelus malabarichus	0 (4)
Ephippus orbis	50 (2)
Etroplus suratensis	100 (1)
Euthynnus affinis	50 (2)
Exocoetus volitons	50 (8)
Gerrus filamentosa	25 (4)
Hemiramphus xanthopterus	33.33 (6)
Ilisha elongata	60 (5)
Lates calcarifer	50 (4)
Leiognathus equulus	24.13 (23)
Mugil cephalus	41.66 (36)
Megalops cyprinoides	100 (1)
Nemipterus japonicus	25 (24)
Parastromateus niger	25 (24)
Pomadassys hasta	33.33 (3)
Psettodes erumeii	31.57 (19)
Rastrilliger kanagurta	31.66 (60)
Sardinella spp.	36.66 (120)
Sciaena dussimieri	66.66 (3)
Scoliodon sorrakowah	25 (8)
Sillago sihama	38.46 (39)
Sphryanera obtusata	25 (8)
Therapon jarbua	100 (1)
Tenulosa sinensis	100 (1)
Total	33.58 (536)

[a] Value in the parenthesis indicates the number of samples analysed.

fishes sold in retail outlets of Switzerland where they reported an extremely high prevalence (95.06%) of *A. hydrophila*. Variations in the incidence level of *A. hydrophila* in the seafood of different parts of the world can be attributed to secondary contamination during handling, storage and transportation. Water has frequently been shown to be contaminated with *Aeromonas* species (Burke et al., 1984b; Slade et al., 1986) and it is likely that contaminated water may have contributed to the high incidence. Of the 278 prawns analysed during the study period, 17.62% of the samples tested positive for *A. hydrophila*. The prevalence levels were highly variable during different months (Table 1). The present investigation showed a higher incidence of *A. hydrophila* in fishes compared to prawns, which is in agreement with the findings of Tsai and Chen (1996).

Table 2 represents the number of different fishes analysed during the study period and the prevalence of *A. hydrophila* in each species. We have analysed more samples of popular table fishes, as they were readily available during most part of the study period. The results revealed that the popular fishes such as sardines, mackerel, mugil and carangids had considerable levels of prevalence (more than 30%) of *A. hydrophila*. The prevalence level varied from 0% to 100% in different fishes. The overall prevalence levels in the popular table fishes were comparable to the levels recorded by Hatha et al. (2000) in the farm raised fresh water fishes.

The prevalence of *A. hydrophila* in different prawn species such as *Penaeus indicus*, *Penaues monodon* and *Penaeus semisulcatus* was found to be 16.58%, 13.20% and 25.52% respectively. The overall prevalence levels in prawns were much lower than those recorded in fishes. This is in agreement with findings of Tsai and Chen (1996). The chitinous shell of the prawns may not be that conducive for proliferation of the *A. hydrophila*, as the moisture rich body surface of fish.

The results of the seasonal variation in the prevalence of *A. hydrophila* in marketed fish and prawns during the study period revealed maximum incidence during the monsoon season followed by post-monsoon and pre-monsoon (Table 3). This could be due to the increased coastal water pollution resulting from land run off, municipal sewage outflows and storm water surge during the monsoon season. It is also reported that high humidity and low temperature is preferred by many

Table 3
Seasonal variation in the prevalence of *A. hydrophila* in fish and prawn during June 1997–May 1999

Year and season	Prevalence (%)	
	Fish	Prawn
1997–98		
Pre-monsoon	23.21 (56)[a]	18.49 (47)[a]
Monsoon	38.46 (91)	20.58 (34)
Post-monsoon	28.73 (87)	14.63 (41)
1998–99		
Pre-monsoon	33.33 (114)	15.51 (58)
Monsoon	41.50 (106)	22.91 (48)
Post-monsoon	30.48 (82)	18.00 (50)

[a] Values in the parenthesis indicate the number of samples analysed during each season.

pathogens (Baker, 1990), which is a well-marked feature during the monsoon season. However, the results were contradictory to the findings of Burke et al. (1984a), who reported higher incidence of *A. hydrophila* in smoked catfish fillets during the summer months. Kaper et al. (1981) also observed higher levels of *A. hydrophila* in Chesapeake Bay during spring season.

The frequency of isolation of *A. hydrophila* from the different parts of the body of fishes analysed. The strains of *A. hydrophila* were frequently isolated from the intestinal region (38.43%) of the fishes followed by body surface (32.46%) and gill (29.10%). Our findings are supported by the observations of Hatha (2002) who has reported that motile aeromonads are a part of the resident microflora of the intestine of farm raised fresh water fishes. *A. hydrophila* has also been isolated from wild fish, and pond cultured edible and ornamental fish (Palumbo et al., 1985; Gobat and Jemmi, 1993). It is also consistently isolated from fishes affected with epizootic ulcerative syndrome from different parts of the world (Snieszko and Bullock, 1976, Rahman et al., 2002).

The transportation of fish and prawns from other states to Coimbatore will take at least one day. Though the foods are carried under iced condition, one of the characteristics that strongly influence the potential importance of *A. hydrophila* in regards to food safety is its psychrotrophic nature. Bergey's manual (Popoff, 1984) highlights *A. hydrophila* as being capable of showing growth over a temperature range from 0°C to 41°C. At refrigeration temperatures (4–7°C), this species grow at a sufficiently rapid rate as to be competitive with other psychrotrophic species associated with foods. Considering the psychrotrophic nature of this organism and the obvious implication of food poisoning strains that can grow readily at refrigeration temperatures, it seems imperative that the information needed to assess their food safety significance be obtained at the earliest possible date. Apart from the psychrotrophic nature of this organism, during the transportation, the periodical dampening of seafood with contaminated water, besides sprinkling with contaminated wet sand and packing it with cheap quality of ice are customary practices to decelerate the tropical heat. Cumulative effect of such conventional practices coupled with unhygienic handling during transportation results in secondary contamination and explains the high prevalence level of *Salmonella* species in marketed seafood (Hatha and Lakshmanaperumalsamy, 1997). Moreover, the major fish market at Coimbatore is situated near the main municipal treatment plant, which could serve as a source for secondary contamination through flies.

The results of the present investigation revealed that contamination of fish and prawns with *A. hydrophila* in the Ukkadam market is considerably high. Although the source of the organism may be ambient environment, secondary contamination during catching, handling and transportation may also contribute for its distribution. The psychrotrophic nature of this organism and the multiplicity of virulence factors add to the significance of this organism in a highly perishable commodity such as seafood. Until more definite information is available concerning this micro-organism in seafoods, it seems prudent to recommend that individuals involved in the catching, storage, distribution and processing of seafoods consider *A. hydrophila* as undesirable.

Aeromonas spp. is being considered as a pathogen of emerging importance due to its special features such as ubiquitous presence in the aquatic environment, multiplicity of virulence factors and psychrotrophic nature. Though Indian cooking processes involve high degree of boiling and extensive use of spices, which might eliminate this organism from the respective seafood, the toxin may remain in the foodstuff. Central institute of Fishery technology (CIFT), Government of India had prescribed a series of guidelines for an ideal fish market, which are to be strictly followed in order to avoid secondary contamination and growth of *A. hydrophila* in seafoods.

Acknowledgements

The authors are thankful to the Head, Department of Environmental Sciences, Bharathiar University, Coimbatore for providing necessary laboratory facilities.

References

Abeyta, C., Wekell, M.M., 1988. Potential sources of *Aeromonas hydrophila*. J. Food Saf. 9, 11–12.

AOAC, 1975. In: Howitz, W. (Ed.), Official Methods of Analyses, 12th Edition. Association of Official Analytical Chemists, Washington.

Ashdown, L.R., Koehler, J.M., 1993. The spectrum of *Aeromonas* associated diarrhea in tropical Queensland, Australia. South Asian J. Trop. Med. Public Health 24, 347–353.

Austin, B., Adams, C., 1996. Fish pathogens. In: Austin, B., Altwegg, M., Gostling, P.J., Joseph, S. (Eds.), The genus *Aeromonas*. Wiley, New York, NY, pp. 197–229.

Baker, R.C., 1990. Survival of *Salmonella enteritidis* on and in shelled eggs, liquid eggs and cooked egg products. Dairy, Food Environ. Sanit. 10, 273–275.

Buchanan, R.L., Palumbo, S.A., 1985. *Aeromonas hydrophila* and *Aeromonas sobria* as potential food poisoning species: a review. J. Food Saf. 7, 15–29.

Burke, V., Robinson, J., Cooper, M., Beaman, J., Partridge, K., Peterson, D., Gracey, M., 1984a. Biotyping and virulence factors in clinical and environmental isolates of *Aeromonas* species. Appl. Environ. Microbiol. 47, 1146–1149.

Burke, V., Robinson, J., Gracey, M., Peterson, D., Meyer, N., Haley, V., 1984b. Isolation of *Aeromonas* spp. from an unchlorinated domestic water supply. Appl. Environ. Microbiol. 48, 367–370.

Day, F., 1889. . The fishes of India; A natural History of the Fishes Known to Inhabit the Seas and Freshwaters of India, Burma and Ceylon, Vol. I and II. Today and Tomorrow's Book Agency, New Delhi.

Fricker, C.R., Tompsett, S., 1989. *Aeromonas* spp. in foods: a significant cause of food poisoning? Int. J. Food Microbiol. 7, 17–23.

Gobat, P.F., Jemmi, T., 1993. Distribution of mesophilic *Aeromonas* species in raw and ready-to-eat fish meat products in Switzerland. Intl. J. Food Microbiol. 20, 117–120.

Granum, P.E., O'Sullivan, K., Tomas J, M., Ormen, O., 1998. Possible virulence factors of *Aeromonas* spp. from food and water. FEMS Immunol. Med. Microbiol. 21, 131–137.

Hatha, A.A.M., 2002. Transitory and resident microflora of the gastrointestinal tract of farm raised fresh water fishes in relation to habitat microflora. Asian J. Microbiol. Biotechnol. Environ. Sci. 4 (2), 277–282.

Hatha, A.A.M., Lakshmanaperumalsamy, P., 1997. Prevalence of *Salmonella* in fish and crustaceans from markets in Coimbatore, South India. Food Microbiol. 12, 111–116.

Hatha, A.A.M., Kuruvilla, S., Cheriyan, S., 2000. Bacterial flora associated with the intestines of farm raised fresh water fishes *Catla catla*, *Labeo rohita* and *Ctenopharyngodon idella*. Fish. Technol. 37 (1), 59–62.

Hudson, J.A., Delacy, K.M., 1991. Incidence of motile aeromonads in New Zealand retail foods. J. Food Prot. 54, 696–699.

Janda, J.M., Abbott, S.L., 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations and unanswered questions. Clin. Infect. Dis. 27, 332–344.

Kaper, J.B., Seidler, R.J., Lockman, H., Colwell, R.R., 1979. Medium for the presumptive identification of *Aeromonas hydrophila* and Enterobacteriaceae. Appl. Environ. Microbiol. 38, 1023–1026.

Kaper, J.B., Lockman, H., Colwell, R.R., 1981. *Aeromonas hydrophila*: ecology and toxigenicity of isolates from an estuary. J. Appl. Bacteriol. 50, 359–377.

Kirov, S.M., Hudson, J.A., Hayward, L.J., Mott, S.J., 1994. Distribution of *Aeromonas hydrophila* hybridization groups and the virulence properties in Australasian clinical and environmental strains. Lett. Appl. Microbiol. 18, 71–73.

Krovacek, K., Pasquale, V., Baloda, S.B., Soprano, V., Conte, M., Dumontet, S., 1994. Comparison of putative virulence factors in *Aeromonas hydrophila* strains isolated from the marine environment and human diarrhoeal cases in south Italy. Appl. Environ. Microbiol. 60, 1379–1382.

Majeed, K.N., Egar, A.F., MacRae, I.C., 1989. Enterotoxigenic aeromonads on retail lamb meat and offal. J. Appl. Bacteriol. 67, 165–170.

Misra, K.S., 1959. An aid to the identification of the common commercial fishes of India and Pakistan. Rec. Ind. Mus. 57, 1–320.

Monfort, P., Baleux, B., 1990. Dynamics of *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae* in sewage treatment pond. Appl. Environ. Microbiol. 56, 1999–2006.

Munro, I.S.R., 1955. The marine and freshwater fishes of Ceylon. Department of External Affairs, Canberra, 349pp.

Okrend, A.J.G., Rose, E., Bennett, B., 1987. Incidence and toxigenicity of *Aeromonas species* in retail poultry, beef and pork. J. Food Prot. 50, 509–513.

Palumbo, S.A., Maxino, F., Williams, A.C., Buchanan R, L., Thayer, D.W., 1985. Starch ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. Appl. Environ. Microbiol. 50, 1027–1030.

Popoff, M., 1984. Genus III *Aeromonas* In: Kluyver, A.J., Van Niel, C.B. (Eds.), Bergey's Manual of Systematic Bacteriology, Vol. 1. Williams and Wilkins, Baltimore, MD, pp. 545–548.

Rahman, M., Colque-Navarro, P., Kuhn, I., Huys, G., Swings, J., Mollby, R., 2002. Identification and characterisation of pathogenic *Aeromonas veronii* biovar *sobria* associated with epizootic ulcerative syndrome in fish in Bangladesh. Appl. Environ. Microbiol. 68 (2), 650–655.

Slade, P.J., Falah, M.A., Al-Ghady, A.M., 1986. Isolation of *Aeromonas hydrophila* from bottled waters and domestic water supplies in Saudi Arabia. J. Food Prot. 49, 471–476.

Snieszko, S.F., Bullock, G.L., 1976. Disease of freshwater fishes caused by bacteria of the genera *Aeromonas*, *Pseudomonas* and *Vibrio*. FDL 40, USKI, FWS, 10pp.

Tirmizi, N.M., 1967. Commercial prawns of West Pakistan. F.A.O. FR: BCSP/67/E/40.

Tsai, G.J., Chen, T.W., 1996. Incidence and toxigenicity of *Aeromonas hydrophila* in seafood. Intl. J. Food Microbiol. 31, 121–131.

Whitehead, P.J.P., 1972. A synopsis of the clupeoid fishes of India. J. Marine Biol. Assoc. India 14, 160–256.