Seafood Safety - Proceedings of the Symposium on Seafood Safety - Status and Strategies, 28-30 May 2002, Cochin, India, 359-363

Clostridium botulinum in Finfish and Shellfish

K.V. LALITHA and P.K. SURENDRAN
Central Institute of Fisheries Technology
P.O. Matsyapuri, Cochin - 682 029, India

Clostridium botulinum, an anaerobic Gram positive spore forming bacterium, is a food safety hazard. The distribution of *C. botulinum* in wild caught and farmed finfish and shellfish in India was investigated. A total of 226 samples of finfish and shellfish were tested. An overall prevalence of 16% was found. The predominant types were type C and D (11%) followed by type B (3%) and type A (1%). Incidence of *C. botulinum* in wild caught and farmed shellfish was 18% and 23%, respectively. The predominant types were type D (13%) followed by type A (8%) and type C (2%).

Key words: Seafood safety, botulism, *Clostridium botulinum*

Clostridium botulinum is the causative agent for the 'botulism' food poisoning in man. It is a Gram positive anaerobic spore forming bacterium, the strains of which are classified into seven types A to G depending on the serological specificity of the neurotoxin produced. Many of the botulism outbreaks recorded in Canada, USA, USSR, Europe, Japan and Iran were traced to the consumption of uncooked, undercooked, smoked, stale or fermented fish and also to seal or whale meat (Sakaguchi, 1979; Hauschild, 1993). *C. botulinum* is distributed widely in freshwater, brackish water and marine environments and fish (Huss, 1980; 1981; Smith, 1990; Hauschild, 1989; Dodds, 1993; Laitha & Gopakumar, 2000). *C. botulinum* type E is the most dominant type prevalent in aquatic environments of temperate areas, while in tropical areas, types C and D predominate.

Botulism associated with consumption of fresh fish has not been a major food safety concern because *C. botulinum* requires anaerobic conditions for growth. However, the use of modified atmosphere and high barrier film packaging, combined with refrigeration to extend shelf life of fresh fish may increase the risk for *C. botulinum* growth and toxigenesis (Huss, 1981; Lindroth & Genigeorgis, 1986; Garcia *et al.*, 1987; Gopal *et al.*, 1990; 1996; Lalitha & Gopakumar, 2001). This concern is because all type E and non-proteolytic type B and F strains (*C. botulinum* group II) can grow at 3.3°C. All type A and proteolytic type B and F (group I) have a minimum growth

temperature of 10°C and type C and D (group III) do not grow below 15°C. Groups I and II are associated with human botulism, while group III is responsible for animal botulism (Hauschild, 1993). However, the potential hazards of types C and D human botulism is clearly indicated (Dolman *et al.*, 1961; Oguma *et al.*, 1990). The aim of the present investigation was to gain information on contamination levels and type distribution of *C. botulinum* in wild caught and farmed fish with a view to assessing the risks associated with seafoods from tropical Indian waters.

Materials and Methods

Two hundred and twenty-six samples of wild caught finfish and shellfish obtained onboard CIFT research vessels operating off Cochin and from the retail markets in and around Cochin and 66 farmed fish and shellfish samples from farms in and around Cochin were included in this study.

Samples were examined for the presence of *C. botulinum* as described by Lalitha & Gopakumar (2000). Samples (3-5 g) were inoculated into 25 ml cooked meat media and sterile paraffin oil was poured above the medium as an overlay. Inoculated tubes were incubated at 30°C for 3-6 days. Toxicity of the supernatant was tested by mouse bioassay as per USFDA (Solomon & Lilly, 1998). Toxin neutralization tests were made using type specific monovalent antitoxins A-F obtained from US Centre for Disease Control (Atlanta, GA). The number of *C. botulinum* present in bivalve, farmed fish and shellfish samples were estimated by the three tube Most Probable Number method using the procedure followed by Cann & Taylor (1984) and Baker *et al.* (1990).

Results and Discussion

Of the total of 226 samples of wild finfish and shellfish tested, *C. botulinum* was detected in 16%. Of the 153 samples of wild finfish examined, the predominant types were type C and D (12%) followed by type B (3%) and type A (1%). In finfish obtained from the retail markets, the overall contamination level was 20% (10/50). Contamination of wild caught freshwater fish and prawn by *C. botulinum* types C and D was 30% (3/10). *C. botulinum* was prevalent in 12% (11/90) of the freshly caught finfish and shellfish. Incidence of *C. botulinum* in wild caught shellfish was 18% (13/73); type D 10% and type C 8%. The number of spores (MPN) of *C. botulinum* in bivalves ranged from 0 - 70.100 g^{-1}.

A total of 66 samples of farmed finfish and shellfish were examined (Tables 1 & 2). Nine samples (23%) harboured *C. botulinum* with a range of MPN 0-150.100g^{-1} (mean 17.100g^{-1}). The predominant type was type D (13%), followed by type A (8%) and type C (2%). A higher frequency (30-38%) has been noticed in bivalves and number of spores was in the order of 0-40.100g^{-1}. In farmed fish, the relative frequency and number of spores were low, with a mean count of 6.100g^{-1} and frequency of 11%.

Table 1. Prevalence of *Clostridium botulinum* in wild caught and farmed fish

Source of sample	No. of samples tested	% positive	Types identified
Wild caught fish			*
Marine species			
Onboard vessel	90	12	D (5), C (3), B (2), A (1)
Cochin retail market	50	20	B (2), C (3), D (4), 1 (not identified)
Brackishwater species			
Cochin retail market	9	22	A (1), C (1)
Freshwater species			
Cochin retail market	4	25	C (1)
Farmed fish			
Brackish water	15	13	C (1), 1 (not identified)
Freshwater	11	9	B (1)

Figures in brackets indicate the number of cultures identified

Clostridium botulinum types C and D are more prevalent in fish from the coastal and inland areas of India. This finding is similar to those made earlier in the tropical waters (Huss, 1980; Hauschild, 1989; 1993). The prevalence of *C. botulinum* (16%) found in this study for wild caught finfish is close to the overall incidence (14-17%) estimated for fish in Indonesia and Iran (Hauschild, 1989). Contamination of wild caught freshwater fish with *C. botulinum* types C, D and E has earlier been reported in Indonesia (14%)(Haq & Suhadi, 1981) and with types C, E and F in Japan (Azuma & Itoh, 1987).

Fresh fish have never been implicated in human botulism (Huss, 1994). The mere presence of *C. botulinum* serotypes in fresh fish will not cause illness. It is necessary that viable *C. botulinum* spores should be present and are given the opportunity to germinate and produce toxin. So far botulism has not been reported in India. Psychrotrophic strains of *C. botulinum* (type

Table 2. Prevalence of *Clostridium botulinum* in wild and farmed shellfish

Source of sample	No. of samples tested	% positive	Types identified
Wild caught shellfish			
Marine species			
Prawn	29	27	C (2), D (6)
Mussel	26	12	C (3)
Cuttlefish and squid	6	0	-
Brackishwater species			
Clams	6	0	-
Freshwater species			
Prawn	6	33	C (1), D (1)
Farmed shellfish			
Brackish water prawn	20	15	C (1), D(1)
Freshwater prawn	2	0	-
Mussel	10	30	A (3)
Oyster	8	38	D (3)

Figures in brackets indicate the number of cultures identified

E and non-proteolytic types B and F) are noticeably absent in finfish and shellfish. The ultimate safeguard is the very low heat stability of botulinum toxin (Huss, 1981; Hauschild, 1989). The preference for 'fresh and fresh like' foods and minimally processed foods has considerably increased in recent years. The risk of botulism can be increased by such fish preservation process as they foster the growth of *C. botulinum* if processing is not proper and as these foods are consumed without cooking. Therefore, the recovery of these pathogens from seafoods emphasizes the need for promoting good sanitation and appropriate methods of preservation to keep the contamination at low level, to prevent toxigenesis and to ward off post process contamination.

References

Azuma, R. and Itoh, T. (1987) in *Avian Botulism - An International Perspective*, (Eklund, M.W. and Dowell Jr., V.R., Eds.), p. 167-187, Springfield, IL, USA

Baker, D.A., Genigeorgis, C. and Garcia, G. (1990) *J. Food Protect.* **53**, 668

Cann, D.C. and Taylor, L.Y. (1984) *J. Fish Dis.* **7**, 391

Dodds, K.L. (1993) in *Clostridium botulinum: Ecology and Control in Foods* (Hauschild, A.H.W. and Dodds, K.L., Eds.), p. 21-68, Marcel Dekker Inc., New York

Dolman, C.E., Darby, G.E. and Lane, R.F. (1961) *J. Infect. Dis.* **109**, 107

Garcia, G., Genigeorgis, C. and Lindroth, S. (1987) *J. Food Protect.* **50**, 330

Gopal, T.K.S., Nambiar, V.N., Bhattacharya, S.K., Joseph, J. and Prabhu, P.V. (1990) in *Proc. Symposium on Chilling and Freezing of New Fish Products*, p. 141-146, International Institute of Refrigeration, London

Gopal, T.K.S., Nambiar, V.N., Lalitha, K.V., Joseph, J. and Prabhu, P.V. (1996) in *Proc. Second Workshop Scient. Results of FORV Sagar Sampada*, (Pillai, V.K., Abidi, S.A.H., Ravindranathan, V., Balachandran, K.K. and Agadi, V.I., Eds.), p. 513-519, Dept. Ocean Development, India

Haq, I. and Suhadi, F. (1981) *Japan J. Med. Sci. Biol.* **34**, 231

Hauschild, A.H.W. (1989) in *Food-borne Bacterial Pathogens*, (Doyle, M.P., Ed.), p. 111-189, Marcel Dekker Inc., New York

Hauschild, A.H.W. (1993) in *Clostridium botulinum: Ecology and Control in Foods*, (Hauschild, A.H.W. and Dodds, K.L., Eds.), p. 69-104, Marcel Dekker Inc., New York

Huss, H.H. (1980) *Appl. Environ. Microbiol.* **39**, 764

Huss, H.H. (1981) *Clostridium botulinum type E and botulism*, Technical Univ. of Denmark, Lyngby, Denmark

Huss, H.H. (1994) *FAO Fisheries Technical Paper* 334, FAO, Rome

Lalitha, K.V. and K. Gopakumar (2000) *Food Microbiology* **17**, 535

Lalitha, K.V. and Gopakumar, K. (2001) *Food Microbiology* **18**, 651

Lindroth, S.E. and Genigeorgis, C.A. (1986) *Int. J. Food Microbiol.* **3**, 167

Oguma, K., Yokota, K., Hayashi, S., Takeshi, K., Kumagai, M., Itoh, N., Tachi, N. and Chiba, S. (1990) *Lancet.* **336**, 1449

Sakaguchi, G. (1979) in *Food borne Infections and Intoxications*, 2nd edn., (Riemann, H. and Bryan, F.L., Eds.), p. 389-442, Academic Press, London

Smith, G.R. (1990) in *Topley and Wilison's Principles of Bacteriology, Virology and Immunity*, Vol. 3 *Bacterial Diseases*, (Smith, G.R. and Easman, C.S.F., Eds.) p. 514-529, Edward Arnold, London

Solomon, H.M. and Lilly, T. Jr. (1998) in *Bacteriological Analytical Manual*, 8th edn., Chapter 17, AOAC International, Gaithersburg, MD, USA