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Occurrence of *Clostridium botulinum* in Fish and Fishery Products in Retail Trade, A Review Article

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Abstract: Most outbreaks of food poisoning associated with fish is derive from the consumption of raw or insufficiently heat treated fish, which may be contaminated with bacteria from water environment (*Vibrio* spp.,*C. botulinum*) or fish products recontaminated after heat processing. Wound infections, caused particularly by mycobacteria are seen after injury during handling fish or after exposure of open wounds to water environment. In the case of poor hygiene, the contamination of fish and fish products may be increased due to unsanitary procedures, the rotation of the assigned duties of workers and airborne microorganisms during packing of the product. Hot smoking in mild conditions at a temperature not exceeding 65°C and a low concentration of salt does not inactivate all pathogens or inhibit bacteria during storage. Thus the required safety can be obtained only by using very fresh fish handled in hygienic conditions, controlling the processing and the plant hygiene at critical control points and chilling of the product to about 2°C. The prevalence of *Clostridium botulinum* type E gene in fish and fishery products of commercial importance is evident in Iran. The results demonstrate that *C. botulinum* type E poses a serious health risk for those consuming fishery products.

Key words: Risk assessment · Food safety · *C. botulinum* · Fish and fishery products

INTRODUCTION

Botulism is a rare, but it causes serious paralytic illness due to a nerve toxin that is produced by the bacterium *Clostridium botulinum*. The illness is extremely rare in New Zealand (there has been only one outbreak involving two individuals affected by the toxin produced by *C.botulinum* Type A). However, it is relatively common in some parts of the world. This bacterium only grow and produce toxin in the absence of oxygen. *C. botulinum* is only of concern in seafood products that have been improperly heated during processing such as bottling or canning or in products that have been packaged under regimes designed to limit the availability of oxygen. *C. botulinum* type E is of most concern to producers of these types of food as it is a naturally occurring marine organism and grow at refrigeration temperatures (as low as 2.9°C). It is therefore able to proliferate in seafood products if they are poorly chilled [1].

Biology of the Organism: *C. botulinum* is an anaerobic, Gram positive, spore-forming bacilli that produces a potent neurotoxin. The spores are heat-resistant and can survive in foods that are inproperly processed. The toxin is heat labile and can be destroyed by adequate cooking. *C. botulinum* is grouped into seven types, A, B, C, D, E, F and G, based on the antigenic specificity of the toxin produced by each strain. Types A, B, E and F cause human botulism. Types C and D caused most cases of botulism in animals. Animals most commonly affected are wild fowl and poultry, cattle, horses and some species of fish. Although type G has been isolated from soil in Argentina, no outbreaks involving it have been reported [2].

Toxin Inactivation: While, the spores of *C. botulinum* are relatively resistant to heat, the toxins are known to be relatively sensitive. Normal cooking procedures usually destroy toxins in foods. Heating food to an internal temperature of 78°C for 1 minute has been suggested as

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a general guide for destroying toxins in convenience foods. However, it has also been reported that both the heating medium and pH markedly affect the inactivation rate of the toxin [3].

Diseases: All three forms of botulism are fatal and are considered of medical emergencies. Food-borne botulism can be especially dangerous because many people can be poisoned by eating a contaminated food [4].

Food-borne Botulism: Food-borne botulism is the disease (actually a food-borne intoxication) caused by the consumption of foods containing the neurotoxin produced by *C. botulinum.* A very small amount (a few nanograms) of toxin can cause illness. Onset of symptoms in food-borne botulism is usually 18 to 36 hours after eating the food containing the toxin, although cases vary from 4 hours to 8 days. Early signs of intoxication are marked lassitude, weakness and vertigo, usually followed by double vision, drooping eyelids and progressive difficulty in speaking and swallowing. Difficulty in breathing, weakness of other muscles, abdominal distension and constipation may also be common. If untreated, the illness can progress to cause descending paralysis of respiratory muscles, arms and legs. The fatality rate is 5-15%. Botulinum antitoxin (not currently held in New Zealand) can prevent the progression of the illness and reduce symptoms in severe cases of botulism if administered early [5].

Sources of Clostridium Botulinum: The organism and its spores are widely distributed in nature. They occur in both cultivated and forest soils, bottom sediments of streams, lakes and coastal waters. They also occur in the intestinal tracts of fish and mammals and in the gills and viscera of crabs and other shellfish. While no longer a new technology, modified atmosphere packaging (MAP) is continually being applied to novel foods and processes, raising safety issues, particularly with respect to *C. botulinum*. The main purpose of MAP processing of foods is to inhibit aerobic spoilage flora. This is done by eliminating or limiting oxygen and adding carbon dioxide. However, these conditions still allow the growth of *C. botulinum*. Therefore, if temperature control is inadequate toxin production can occur in the absence of obvious signs of spoilage that might otherwise alert the consumer to the fact that the food is potentially unsafe. MAP seafood products must be held at temperatures below 2.8°C to ensure food safety. If this cannot be guaranteed over the shelf life of the product then additional preservative measures such as salting, smoking, hot processing or the addition of preservatives such as nitrite must be used [6].

Control of Clostridium Botulinum inMAP Packaged Seafoods: Non-proteolytic *C. botulinum* type E, which is able to grow at temperatures as low as 2.9°C is of most concern to producers of MAP packaged seafood products. This bacterium has not been isolated from the New Zealand marine or terrestrial environment so it is difficult to clearly define the risk it poses for New Zealand seafood products. However, the absence of any incidences of botulism associated with MAP seafood products to date suggests that the risk is very low. Nevertheless, seafood processors should adopt strict regimes to limit potential contamination and ensure their products are adequately chilled [7].

The increasing use of vacuum-packaging technology for fishery products, combined with chill storage to extend shelf-life, has raised concerns about the potential health risk for consumers caused by nonproteolytic *C. botulinum.* The processing of most hot- and cold-smoked and raw pickled vacuum-packed fishery products with minimal or no preservatives is insufficient to inactivate clostridial spores; however, the predominant spoilage microorganisms are selectively eliminated or their growth inhibited [6]. The non-proteolytic toxin types of *C. botulinum* are psychrotrophic organisms which are capable of growth at low temperatures [7]. Contamination of live fish by *C. botulinum* has been recognized for many years. Worldwide prevalence studies in temperate geographical areas have shown *C. botulinum* type E as the most prevalent toxin type in aquatic environments and in fish and fishery products [8-11]. These studies have also documented the regular isolation of proteolytic types A, B and F and nonproteolytic types B and F, but the prevalence of these serotypes have been found to be considerably lower than that of type E. Type E has been implicated in the majority of botulism cases associated with the consumption of fish and fishery products [11-13]. Baltic Sea coastal waters are heavily contaminated with *C. botulinum* type E. Various surveys have shown a prevalence close to 100% in marine, freshwater and trout farm sediment samples in Denmark and Sweden [10, 14-16]. High contamination levels have also been reported in various fish species caught in the Baltic Sea near the Danish and Swedish coasts [14, 17, 18] as well as in trout originating in Danish trout farms [16]. A recent survey detected a high prevalence of *C. botulinum* type E in Finnish marine and freshwater bottom sediments [19]. No other toxin types were demonstrated. Knowledge of the contamination level of Finnish fish is limited to a small scale survey of two trout farms [20], in which 4 and 10% of the intestinal samples of rainbow trout were positive. No other fish species have been studied. Very few data are available on the prevalence of type E in vacuum-packed fishery products and fish reaching the consumer in Europe. In the United Kingdom, Cann DC *et al*. [21] detected a type E contamination level of 0-77%in vacuum-packed fishery products. Due to known heavy contamination of Baltic Sea sediments we had reason to expect a considerably higher prevalence in fishery products originating from this area.

Understanding of the prevalence and properties of the organism is essential when considering the safety of fishery products, especially where new or modified preservation methods must be evaluated. In view of the results of the earlier bottom sediment distribution study only *C. botulinum* type E prevalence was examined [19].

A moderately high prevalence of *C. botulinum* type E in Finnish non-farmed fish was detected in this survey. Earlier studies conducted in the Baltic Sea area [14, 17, 18] had shown variable prevalences depending on the fish species examined, but in general the contamination level has been found to be high. There seems to be a considerable difference in the contamination levels of marine and freshwater fish. Definite conclusions, however, should be avoided, since a comparison of marine and freshwater contamination levels was not made within a single fish species. The feeding habits of the fish appeared to have an in-uence on the level of contamination. The lowest prevalence was recorded for vendace, which feed only on plankton. Of the other non-farmed fish species studied, burbot is a predatory fish and whitefish a bottom feeder. During the early stage of life Baltic herring is a plankton feeder, but later it also feeds at the bottom on crustaceans and fish fry [22].

C. botulinum type E appeared to be slightly more prevalent in fish surface samples than in the intestines, but the difference was not statistically significant. It has been reported [23] a considerably higher prevalence of type E in bottom-feeding fish species as compared to planktonfeeders; they concluded that the sea bed was the primary source of contamination.. The prevalence of *C. botulinum* type E did not differ greatly between wild and farmed fish. Nor was there any difference in contamination levels between freshwater and marine trout farms. A study of four Danish freshwater trout farms [24] showed a very high type E prevalence of 65% in whole rainbow trout. A considerably lower contamination level of 11% was detected in Norwegian freshwater trout farms [25]. There is no information available on the situation in marine farms. The variance in contamination levels between different countries may be due to differences in fish farm construction and feeding systems. One potential source of contamination is Baltic herring, which is widely used as additional feed in rainbow trout farms in Finland. The presence of *C. botulinum* type E was also demonstrated in the Finnish fish roe and fishery products, although the numbers of spores found were generally lower than in the raw fish samples. The reason for the high contamination level of burbot roe remains obscure. The finding is especially peculiar in that the sampling was mostly performed directly from the intact roe pouch of fresh burbot. The roe samples of the other fish species were mainly frozen and salted. One possible contamination route of the roe pouch is through the urogenital opening, which lies just next to the anus of the fish. The temperature applied in current commercial hot-smoking treatments is generally insufficient to eliminate natural contamination of fish with *C. botulinum* type E spores. The temperature of the deep fish usually increases to 60-80°C and remains at this level for approximately 30 min. The heat resistance of the spores is increased by a high fat and protein content, brining and a dry environment [26-28]. All these factors are usually present in a fishery product. With the exception of the Baltic Sea area, the European levels of contamination of processed fish by type E appear to be much lower than levels in the USA [2, 21, 29, 30]. Surveys from the Baltic countries report prevalences of 13 and 20% in Swedish hot-smoked Baltic herring [31] and Lalitha, [32], respectively. found type E in 5% of hot-smoked farmed trout. The contamination levels of raw fish detected in the present study seemed to have little effect on the contamination levels of processed fishery products. *C. botulinum* type E gene could not be demonstrated in hot-smoked Baltic herring samples, although fresh herrings were heavily contaminated. This could be due to the hot-smoking process for herrings, during which temperatures high enough to inactivate botulinum spores are found throughout the small-sized product. In this study, vacuum-packed hot-smoked whitefish products were associated with a fairly high botulism risk. The processing method for this product probably contributed to the high contamination level. Eviscerated whitefish are typically smoked whole. Spores are likely to survive better in the gills or on the peritoneum of a large whole fish during the hot smoking process than for example on the surface of a fillet. In addition, the spore load of a fillet would be lower after proper cleaning. The hot-smoking process also eliminates competing micro-ora and may give a heat shock to the botulinum spores, which is not the case for cold-smoked or raw pickled products. All the positive hot-smoked whitefish samples originated from the same plant. This could have been due to the sampling, as 36% of the whitefish products studied came from this plant. Alternatively it could have been an indication of a problem in the manufacturing process of this plant. Spores brought into the plant along with raw fish could have contaminated the production environment. The sodium benzoate used as a preservative in the hot-smoked products of the plant may have further inhibited the growth of competing micro-ora. The hot-smoking process used at the plant may have favored the survival of spores, for example, if the relative humidity applied during the heat treatment was low. The results of this survey show that *C. botulinum* type E is prevalent in many fish species caught from the Baltic Sea, posing a health risk for consumers and an economic risk for the fishing industry, as shown by two Swedish outbreaks during the 1990s [3, 4]. In most foods, botulinum spores are of no consequence unless they are able to grow and produce toxin. At present, the inhibition of botulinum toxigenesis in fishery products relies almost solely on NaCl and refrigeration below 3°C. It is questionable whether this strict temperature requirement is achievable in retail and domestic refrigeration. There would be good reason to limit the shelf-lives of vacuum-packed fishery products and to re-introduce the use of nitrite as an antibotulinogenic substance in fishery products [33- 35]. The development of reliable time-temperature indicators for refrigerated long-storage fishery products would do much to restore customer confidence in these products.

In conclusion, the required safety can be obtained only by using very fresh fish handled in hygienic conditions, controlling the processing and the plant hygiene at critical control points and chilling of the product to about 2°C.

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