

Isolation of *Clostridium botulinum* (Types A, B, E) in Sediments from Coastal Areas in the North of Iran

*HR Tavakoli¹, V Razavilar²

¹Dept. of Nutrition and Food Hygiene, School of public health, Baghyatollah University of Medical Sciences, Tehran, Iran

²Dept. of Food Hygiene, School of Veterinary Medicine, Tehran University, Iran

Abstract

Clostridium botulinum has long been recognized as an etiological agent of food borne botulism and has been reported as an important food safety hazard. The aim of this study was to obtain information about *C. botulinum* distribution in north areas sediments of Iran in order to ascertain the risks associated with consumption and processing of fish from these waters. Two hundred and seventy samples of sediments from coastal areas of "Gilan" and "Mazandaran" provinces of Iran were collected and analyzed. The supernatants were inoculated into cooked meat media and then, grown specimens were stained and checked microscopically. After centrifuge, the supernatants were divided into three (untreated, heated, and trypsinised) and toxicity of them were tested by mouse bioassay. All mice controlled for 4 days for symptoms of botulism. Monovalent standard antitoxins were used for detection of toxin type. The present study revealed that the prevalence of *C. botulinum* (types A, B and E) in sediments from different areas of Gilan and Mazandaran was 3.6% and 4.6% respectively, Mean prevalence of *C. botulinum* in sediments from north regions of Iran was 4.1%. It is also demonstrated that *C. botulinum* type E is predominant type seen in aquatic environments of the coastal areas of Iran. This is the first report of *C. botulinum* distribution in the sediments coastal areas of Iran.

KeyWords: *Clostridium botulinum*, Sediments, Food safety, Food poisoning, Aquatic environment, Iran

Introduction

Clostridium botulinum is a Gram-positive sporeforming, rod-shaped bacteria which is obligate anaerobes and produces a protein with a characteristic neurotoxicity. This bacterium has been shown to be distributed widely in soil, sediments and aquatic environments and has been reported as an important food safety hazard (1, 2). There are several botulism hazards associated with the marine environments, sea foods and fish products (3). Nearly all of the fish-borne botulism outbreaks reported in Canada, USA, USSR, Europe, Japan, and Iran have been linked to the consumption of smoked, salt-dried, canned, fermented, and packaged fish which usually eaten without further cooking (3, 4). Although Botulism is rare, fortunately, the disease is severe with a high mortality (5, 6). The US food industry insists that foods involved in botulism (sausages, and other meats, fish, vegetables, and fruit products) be handled with extreme care. Neither the

organism nor its spores are harmful, but the toxin produced during the growth of the organism under anaerobic conditions, is very lethal. The toxin can be destroyed if heated (80° C for 10 min). Seven immunogenic types (A to G) of the toxin have been identified. They represent the most potent poisons known, and have neuro, entero and hemotoxic properties. Botulinal toxin acts by blocking the release of the neuro-transmitter, acetylcholine, which under normal circumstances triggers contractions of the skeletal muscle. The action of botulinum toxin involved three stages includes binding the heavy chain of the toxin to an unidentified receptor of the nerve, translocation of the light chain portion into the nerve interior, and lytic stage which is nerve-activity dependent, and culminating in the blockage of transmitter release (5, 6, 7). *Clostridium botulinum* in fish and the environment shows a strong association. The different types of *C. botulinum* have been isolated from soils,

sea sediments, and aquatic environments in many countries (1, 9, 10, 11). The present study is the first report of *C. botulinum* distribution in the sediments coastal areas of Iran and the aim of this study was to obtain informations on *C. botulinum* distribution in sediment of Caspian Sea in order to ascertain the risks associated with consumption and processing of fish from these waters.

Materials and Methods

Two hundred and seventy samples of sediments were collected from coastal areas of Caspian sea including 140 samples from Gilan province (Hassanrod, Rasht, Lahijan, and Anzali) and 130 samples from Mazandaran province (Ramsar, Noshahr, Sari, and Noor). Samples were collected at a minimum of four different points in the pond in sterile plastic pouches and transported to the lab in an ice box. Suspension of samples were prepared and centrifuged at 5000 rpm for 10 min. The supernatants were inoculated into 25 ml cooked meat media, prepared in the laboratory according to method outlined by FDA (7) and sterile paraffin oil was added to inoculated tubes and incubated for 3-5 days in 30°C. After incubation period, cultures were evaluated macroscopically (turbidity, gas production, and meat digestion) and microscopically (gram staining and observation of sub-terminal spores). Cultures were centrifuged (4°C for 20 min) and each supernatant was adjusted to pH 6.2 within

HCl and frozen at -20° C until tested for the presence of *C.botulinum* toxin. Toxicity of the supernatants was tested by mouse bioassay. All mice were observed periodically for 96 h for symptoms of botulism and death. Typical botulism symptoms in mice in sequence are ruffling of the fur and labored but not rapid breathing, weakness of the limbs, gasping for breath (opening of lower jaw), and death due to respiratory failure (5, 6, 8). In this study we used from reference strains of *C.botulinum* (blanks) including strains of type A (53 A), type B (90 B) and type E (10660 E) and detection of *C.botulinum* toxin type was made using standard Monovalent antitoxin A, B, and E obtained from Pasteur institute, France.

Results

The distribution of *C. botulinum* types in sediment samples from various sources is shown in table 1. The prevalence of *C. botulinum* (types A, B & E) in sediment samples of Gilan and Mazandaran states was 3.6% and 4.6%, respectively, and mean prevalence of *C botulinum* in sediments from those areas was 4.1%. The identified *C. botulinum* types in the Gilan sediments included type E (1.4 %), type A(0.7%) ,and type B(1.4%). Mazandaran sediments included type E (3.1%) , type A (0.8%) and type B (0.8%).The current serotypes isolation of coastal areas sediments of these regions are shown in table 1.

Table 1: Prevalence of *Clostridium botulinum* in sediment samples from coastal areas of Gilan and Mazandaran provinces.

Source of sediment	No. of samples examined	No. of positive samples (%)	Type
Hassanrod	40	-(0)	-
Rasht	40	2(5)	E, B
Lahijan	40	1(2.5)	A
Anzali	20	2(10)	E, B
Ramsar	30	2(6.7)	E, B
Noshahr	30	-(0)	-
Sari	40	3(7.5)	E
Noor	30	1(3.3)	A
Total	310	11(3.54)	A, B, E

Discussion

C.botulinum has been reported as an important food safety hazard and its different types (A, B, C, D) have been isolated from the sediments and coastal areas of many countries (1, 9, 10, 11). *C.botulinum* type E is the most dominant spore type prevalent in sea sediments and aquatic environments. This type is current type in temperate areas of India, whereas types C and D are dominant in tropical areas of this country (1, 2). In Japan soil samples from 98 sites of four rivers were examined for the presence of *C. botulinum*. Type E was prevalently shown throughout the whole river systems and its toxin was detected from 33 to 82% and types C and B toxins were detected from 9% and 7% of the sites examined, respectively. This study suggests that the terrestrial origin of type E organism would be considered as one of the reasons for the high incidence of this organism in the sea areas of Japan (9). The distribution of *C. botulinum* in the natural environments of Denmark, the Faroe, Islands, Iceland, Greenland, and Bangladesh was examined. A total of 684 samples were tested. Type E was found in 90% of samples from the aquatic environments of Denmark and in 86% of samples from the marine environments of Greenland. *C.botulinum* types A, B, and E were found in 26% of samples from the environments of the Faroe Islands, Iceland, and Bangladesh, whereas types C and D were demonstrated in 4.2% of examined samples. It was suggested that type E was a true aquatic organism in Denmark and Greenland natural environments, because this environment offers the best conditions for survival of the spore in nature (10). Based on a study in Scandinavian waters in the North Sea and the North Atlantic, only type E was detected that suggested type E may originate in the sea bed and that they be spreaded by fish and water currents (11). In the present study *C.botulinum* types A, B, and E isolated from the sea sediments and aquatic environments of North of Iran, and the obtained results coincide with results of other studies in different countries which show different types

of *C.botulinum* are exists in soils, sea sediments, and aquatic environments, and *C. botulinum* type E is the most current type in these places. The potential hazards of types A,B and E due to consumption of raw, salted, smoked, and packaged fishery products and food conserves is clearly indicated (1, 4, 6, 8), so there is the risk of extended storage of raw or mildly thermally processed sea foods and is necessary to protect these products from temperature abuse until their final use.

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