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Food safety and botulinum toxin

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Abstract

The botulism is a food intoxication (poisoning) caused by ingestion of preformed toxin produced by C. botulinum that may be ubiquitously found in soil. Tins, smoked dried or vacuum packed dried meat and vacuum packed fish as well as tins acquired from stores are involved. This paper is a retrospective study of food-borne disease with botulinum toxin from 2003 to 2008.
Incidence of botulism in Romania was investigated using blood serum samples collected from suspected patients. The tests were performed in the National Institute of Research and Development for Microbiology and Immunology "I. Cantacuzino", from 2003 to May 2008. In our study mainly botulinum toxin type B was identified, and just in only one case serotype E was found.
Botulinum toxin from blood serum samples was identified in 80 cases: 79 samples were of type B and one sample of type E. Food-borne botulism cases in Romania during the period 2003-2008 were as follows: 27 cases (13 %) in 2003, 18 cases (9%) in 2004, 21 cases (10%) in 2005, 23 cases (11%) in 2006, 110 cases (52%) in 2007, and 11 cases (5%) in 2008.

Keywords: food-borne infection, bacteriology, epidemiology, food industry, *Clostridium botulinum*.

Introduction

Botulism is a food poisoning caused by ingestion of preformed toxin produced by *Clostridium botulinum*, an endo***spore-forming*** bacillus, strictly anaerobic that may be ubiquitously found in soil. Tins, smoked dried or vacuum packaged dried meat and vacuum packaged fish as well as tins acquired from stores can be involved in food-borne botulism [1, 2]. Botulinum toxin like *Clostridium tetani* toxin is a metalloprotein with zinc that binds to the synapses of motor neurons. The preformed botulinum toxin absorbed from gut blocks acetylcholine release at neuromuscular junction and as a consequence produces clinical symptoms [3]. Children botulism and wounds botulism (e.g. intravenous narcotic drug users) are of different pathogenetic mechanism because endospores ingested or inoculated can produce the toxin *in vivo* if they germinate [1].

Symptoms of diseases appear at 18-24 hours after ingestion of toxin and clinical

features are diplodia, dysphonia, dysphagia, difficulty in swallowing and speaking, muscle weakness, nausea, and vomiting [3]. Blepharoptosis, paralysis of the cranial nerves affecting the extra ocular muscles and fixed mydriasis represent characteristic signs. The patient keeps his consciousness. The sensorial organs keep their functionality and the temperature is normal [1]. The respiratory paralysis may cause death if mechanical ventilation is not performed. Treatment requires cleaning the airway, ventilation, and intravenous polyvalent antitoxin because without treatment, 1/3 of the patients may die within a few days of either respiratory or cardiac failure [3].

The most frequently botulinum toxins incriminated in Romanian food poisonings were: type A, B and E. The pH minimum for *C. botulinum* germination, growth and toxin production is 4.5. The minimum water activity (a_w) is 0.94 for types A and B, and 0.97 for type E. Also, heat-resistance is variable from type to type. The heat resistance of botulinum toxins type A and B is higher than type E. Thus, D 110ºC = 2.72-2.82 minutes for type A; D110ºC=1.34-1.37 minutes for type B, and D80ºC=0.80 minutes for type E [4].

Materials and methods

The study was performed on 211 samples of food products collected from 2003 to 2008. Methods of trypsinisation were similar with that of others previously described [5], and toxicity testing of botulinum toxin used in our study was described previously [5, 6, 7]. The sampling was performed as an extraction of a relevant food product. The sample quantity was than divided in three parts: one for detecting viable *C. botulinum* cells, the second for testing the toxicity, and the third part of the sample was kept in the refrigerator. Samples containing solid particles in suspension were centrifuged under refrigeration. The supernatant was used for toxin determination. Solid food samples were extracted by an equal volume of phosphate buffer (pH 6.2). A small quantity of buffer was used in order to avoid the toxin dilution. In our study Thioglycolate Peptone Glucose Yeast Extract ***Medium (***TPGY medium) with trypsin was preferred, because it eliminates other treatments that may degrade the already activated toxin. The pH of medium was adjusted to 6.2 by addition of NaOH 1N or HCl. Then 0.3 ml saturated aqueous trypsin solution was added to 1,8 ml of each supernatant solution (samples for toxicity testing). The solution was then tested for toxicity. (Preparation of the trypsin solution: 1 g trypsin Difco 1/250 was introduced in a sterile culture tube, 10 ml of distilled water was added, was mixed by shaking and heated). The trypsinised sample was incubated at 35-37ºC for 1 hour.

Results and discussion

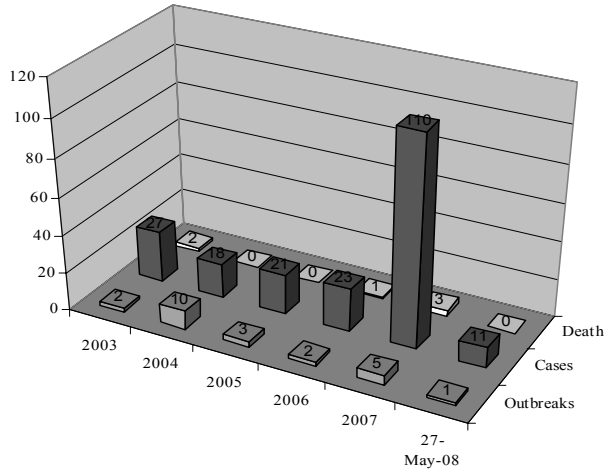
C. botulinum crescend on a worldwide scale and cases of botulism may be met anywhere, but botulism is difficult to diagnose, and the toxin is not always detected in food samples [8]. The botulinum toxin type A occurs more frequently in soils in United States, and toxin type B is found more frequently in Europe. The Baltic Sea displays the highest level of type E contamination in the world [9].

Table 1. Incidence of *C. botulinum* serotype in blood serum in the period 2003-2008, in Romania

Country	Product	Positive samples/ tested samples	*C. botulinum* type (No)	No/g
Romania	Blood serum	80/167	B (79) E (1)	0,0081

Source: *National Institute of Research and Development for Microbiology and Immunology "I. Cantacuzino"*

Another objective of our study was the evaluation of botulism outbreaks in humans (Fig. 1). We noticed that the highest incidence of the cases appeared in 2007 with 110 positive samples. The type of botulinum toxins isolated from food was mainly represented by serotype B. The type E was isolated from fish (Fig. 3).



Source: *National Institute of Research and Development for Microbiology and Immunology "I. Cantacuzino"*
Figure. 1 Epidemic cases and death caused by *C. botulinum* in Romania during the period 2003 to 2008

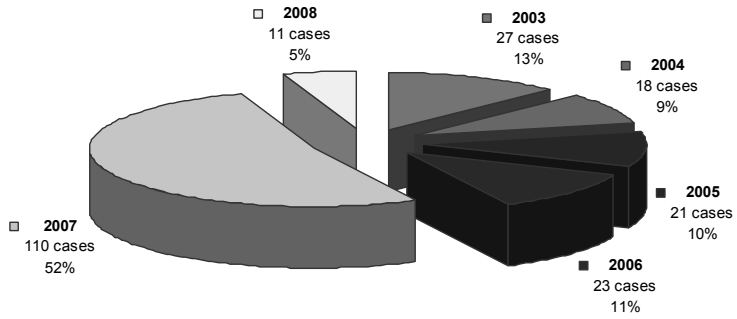
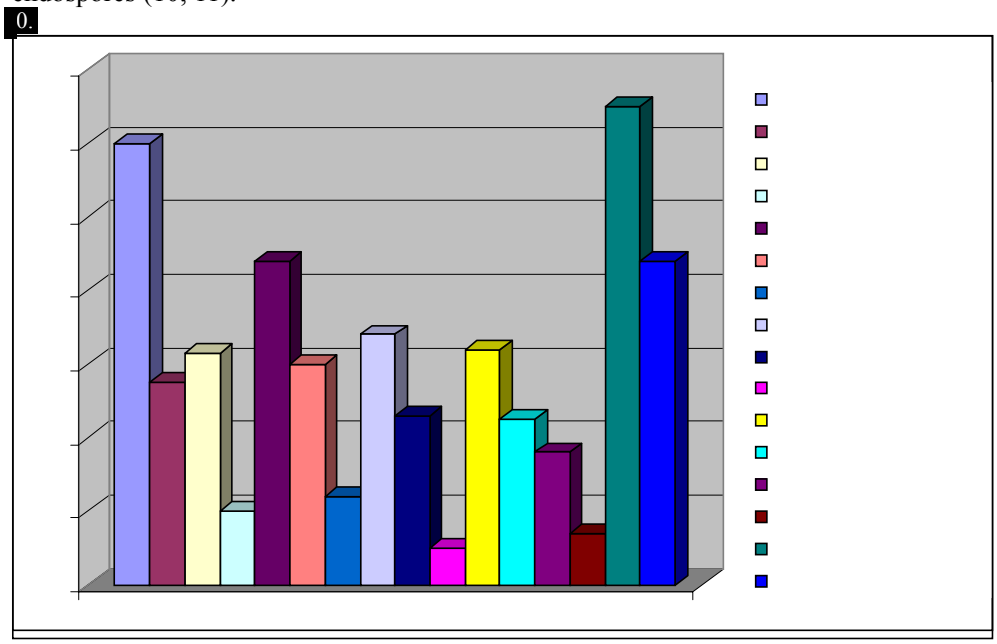


Figure 2. Distribution of botulism cases in Romania during the period 2003-2008

Product	Number Positive/Tested	*C. botulinum* type (no.)	No/g
Tuna fish tins	2/14	B (2)	0,0081
Fish/tuna	2/16	E (2)	0,0092
Bacon	0/16	-	-
Smoked-dried meat	1/16	B (1)	0,0017
Pork	0/7	-	-
Chicken legs	0/5	-	-
Ice-cream	0/6	-	-
Rind	0/7	-	-
Raw sausage	2/16	B (2)	-
Pie	1/15	B (1)	0,0036

Source: *National Institute of Research and Development for Microbiology and Immunology "I. Cantacuzino"*
Table 2. Incidence of botulinum toxins in food products from 2007 to 2008 in Romania

Food products incriminated in food-borne botulism were home-made tins of meat, liver, fish or vegetables, chicken products, pork, fishes and other marine species, respectively semi-tins of fish and tins without an adequate thermal treatment. The scope of the heat treatment is to destroy the botulinum toxin. Honey can contain spores of *C. botulinum* and can be a source for children botulism [8]. In our study this product was free of *C. botulinum* endospores (10, 11).



Source: *National Institute of Research and Development for Microbiology and Immunology "I. Cantacuzino"*
Figure 3. Sources of food-borne botulism during the period 2003-2008, in Romania

Conclusions

1. Botulinum toxin from blood serum samples was identified in 80 cases: 79 samples were of type B and one sample of type E. Food-borne botulism cases in Romania during the period 2003-2008 were as follows: 27 cases (13 %) in 2003, 18 cases (9%) in 2004, 21 cases (10%) in 2005, 23 cases (11%) in 2006, 110 cases (52%) in 2007, and 11 cases (5%) in 2008.
2. In food samples tested during the period 2003-2008 was mainly isolated the serotype B (in 6 cases). Serotype E was found in 2 samples.
3. Foods responsible for induction of food-borne botulism in Romania were the followings: tins, tuna tins, pie, fish, bacon, grated parmesan, sausages, smoked dried meat, carrots, pork meat, chicken legs, ice-cream, rind, pork salami, pork sausage.
4. In our study the following risk factors of *C. botulinum* food-borne disease were identified:
   • Uncontrolled conditions of meat storage and refrigeration (meat storage at 0 ºC or frozen);
   • Inadequate heat treatment to destroy both vegetative and endospore forms of *C. botulinum* (taking into consideration that spore are mesophyl). The suspected food must be boiled at 100ºC for few minutes to be sure that the botulinum toxin was destroyed;
   • Inadequate food preparation (at lower temperatures, when the temperature is not so high to inactivate the spores);
   • Inadequate hygienic conditions (for food safety a perfect hygiene is necessary).

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