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Review

Clostridium botulinum in the post-genomic era

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ABSTRACT

Foodborne botulism is a severe neuroparalytic disease caused by consumption of botulinum neurotoxin formed by strains of proteolytic *Clostridium botulinum* and non-proteolytic *C. botulinum* during their growth in food. The botulinum neurotoxin is the most potent substance known, with as little as 30–100 ng potentially fatal, and consumption of just a few milligrams of neurotoxin-containing food is likely to be sufficient to cause illness and potentially death. In order to minimise the foodborne botulism hazard, it is necessary to extend understanding of the biology of these bacteria. This process has been recently advanced by genome sequencing and subsequent analysis. In addition to neurotoxin formation, endospore formation is also critical to the success of proteolytic *C. botulinum* and non-proteolytic *C. botulinum* as foodborne pathogens. The endospores are highly resistant, and enable survival of adverse treatments such as heating. To better control the botulinum neurotoxin-forming clostridia, it is important to understand spore resistance mechanisms, and the physiological processes involved in germination and lag phase during recovery from this dormant state.

1. Introduction

Clostridium botulinum forms the highly potent botulinum neurotoxin that is responsible for botulism, a severe disease with a high fatality rate. There are three major types of botulism in humans, foodborne botulism, infant/intestinal (adult) botulism and wound botulism. Foodborne botulism is an intoxication caused by consumption of pre-formed toxin, while infant/intestinal (adult) botulism and wound botulism are infections involving toxin formation *in situ*. The symptoms of botulism are primarily neurological and frequently commence with blurred vision, leading to a descending bilateral flaccid paralysis, and in severe cases a flaccid paralysis of the respiratory or cardiac muscles. In many countries, equine antitoxin is administered to adults suffering from botulism, although in severe cases full recovery may take months or even years. The fatality rate is approximately 5–10% of cases. The economic and medical costs associated with foodborne botulism are extremely high (Setlow and Johnson, 1997).

The name "botulism" is derived from the Latin word "*botulus*" meaning sausage, and came to be used in central Europe in the eighteenth century to describe a disease associated with muscle paralysis, breathing difficulties and a high fatality rate that was frequently linked to consumption of blood sausage. A botulism outbreak in Ellezelles (Belgium) in 1895 involving home-made raw salted ham was investigated by Emile van Ermengem, who concluded that "it is highly probable that the poison in the ham was produced by anaerobic growth of specific microorganisms during the salting process" (van Ermengem, 1979; Pickett, 2008). Furthermore, he established that foodborne botulism was an intoxication, not an infection, and that the toxin was produced by a spore-forming obligately anaerobic bacterium. Although these cultures are now lost, their physiological properties are consistent with those of non-proteolytic *C. botulinum* type B. A great number of botulism outbreaks over the next few decades in Europe and North America were associated with the wider use of canning and bottling processes to extend shelf-life. This included, in August 1922, the first recorded outbreak of foodborne botulism in the UK at Loch Maree associated with consumption of sandwiches containing (under-processed) wild duck paste (Leighton, 1923). Identification of the standard minimum heat treatment (known as the botulinum cook) given to low acid canned foods and its correct application then led to a substantial reduction in the number of botulism outbreaks. The prevention of future outbreaks of foodborne botulism will depend on ensuring that known effective control measures (e.g. botulinum cook) continue to be applied correctly, and that appropriate control measures are identified and applied when new processing technologies are introduced and when new types of foods such as minimally heated chilled foods are developed (Peck and Stringer, 2005; Peck et al., 2008). Recommendations on specific control measures for the safe production of minimally heated chilled foods are given in Table 1.

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Table 1
Recommendations on specific control measures to be used for the safe production of minimally heated chilled foods with respect to *Clostridium botulinum* (ACMSF, 2007·FSA, 2008·Peck et al. 2008).

	Specific control measure
(1)	Storage at <3.0 °C
(2)	Storage at ≤8 °C and a shelf-life of ≤10 days (the "10 day rule")
(3)	A heat treatment of 90 °C for 10 min or equivalent lethality (e.g. 80 °C for 129 min, 85 °C for 36 min) combined with storage at chill temperature (designed to give a 6D process for non-proteolytic *C. botulinum*)
(4)	A pH≤5.0 throughout the food, combined with storage at chilled temperature
(5)	A NaCl concentration ≥3.5% throughout the food, combined with storage at chilled temperature
(6)	An a_w≤0.97 throughout the food, combined with storage at chilled temperature
(7)	Combinations of heat treatment and other preservative factors which can be shown consistently to prevent growth and toxin production by *C. botulinum,* combined with storage at chilled temperature[a]

[a] Heat treatments less than 90 °C for 10 min (or equivalents) can be effectively combined with storage temperature and shelf-life to prevent toxin formation from 10^6 spores of non-proteolytic *C. botulinum* (i.e. provide a 6-log process for non-proteolytic *C. botulinum*). For example, heating at 80 °C for 11 min prevented toxin formation at 8 °C in 20 days, while heating at 80 °C for 98 min prevented toxin formation at 8 °C in 40 days (Fernandez and Peck, 1999). Effective combinations of heat treatment, pH, NaCl concentration and shelf-life are also described (Peck and Stringer, 2005).

In 1999/2000, more than 2500 cases of foodborne botulism were reported in Europe, with a high incidence reported in Armenia, Azerbaijan, Belarus, Georgia, Poland, Russia, Turkey and Uzbekistan (Peck, 2006). A smaller, but significant number of cases are reported annually in France, Germany, Italy, China and USA (Peck, 2006). It should be noted that the true incidence of foodborne botulism is likely to be much higher, with under-reporting an issue. Foodborne botulism is not reportable in all countries and the efficiency of investigating potential outbreaks also varies from country to country (Therre, 1999). Outbreaks of foodborne botulism have involved both home-prepared foods and commercially-prepared foods, with proteolytic *C. botulinum* or non-proteolytic *C. botulinum* most frequently implicated (and more rarely neurotoxin-forming strains of *Clostridium baratii* and *Clostridium butyricum*). It should be recognised that since proteolytic *C. botulinum* and non-proteolytic *C. botulinum* are physiologically distinct they present different hazard scenarios.

Foodborne botulism involving proteolytic *C. botulinum* most frequently involves type A or type B neurotoxin. A failure to correctly deliver the botulinum cook (121 °C/3 min) to canned or bottled foods has led to several outbreaks of foodborne botulism (Table 2). In March 2006, a very large outbreak involving 209 cases (134 hospitalised, 42 required mechanical ventilation) was associated with consumption of home-canned bamboo shoots in Thailand (Ungchusak et al. 2007; Wongtanate et al. 2007). An inadequate heat treatment was given, permitting survival of spores of proteolytic *C. botulinum* type A, that was followed by growth and neurotoxin formation during ambient storage. In June 2007, inadequate thermal processing of cans of hot dog chilli sauce at a commercial canning facility in Augusta (Georgia, USA) led to four cases of foodborne botulism (all required mechanical ventilation), and the recall of tens of millions of cans (CDC, 2007). Temperature abuse of foods intended to be stored chilled has also been associated with foodborne botulinum (Table 2). In 2006, a severe outbreak in Canada and USA was associated with commercial chilled carrot juice. It is likely that a very large amount of type A toxin was consumed by affected individuals, since only 5 μl (that contained 7×10^5 MLD_{50}/ml (Sheth et al. 2008)) may have constituted a fatal dose. Six people were affected; all required mechanical ventilation, one of whom died, and two were still dependent on mechanical ventilation one year after the initial intoxication (CDC, 2006; Sheth et al. 2008). In August 2008, a severe outbreak in France was associated with temperature abused commercial chicken enchiladas (Table 2). It is estimated that just 10 mg of food may have constituted a lethal dose (the food

Table 2
Examples of recent incidents of foodborne botulism.

Year	Country	Product	Bacterium	Toxin type	Cases (deaths)	Reference
2000	France	Home-made asparagus soup	Proteolytic *C. botulinum*	B	9	Abgueguen et al. (2003)
2001	USA	Commercial frozen chilli sauce	Proteolytic *C. botulinum*	A	16	Kalluri et al. (2003)
2001	Australia	Reheated chicken	Non-proteolytic *C. botulinum*	E	1	Mackle et al. (2001)
2001	USA	Home-made fermented beaver tail and paw	Non-proteolytic *C. botulinum*	E	3	CDC (2001)
2001	Canada	Home-made fermented salmon roe (2 outbreaks)	Non-proteolytic *C. botulinum*	E	4	Anon (2002)
2002	South Africa	Commercial tinned pilchards	Proteolytic *C. botulinum*	A	2(2)	Frean et al. (2004)
2002	Canada	Restaurant, baked potato in aluminium foil	Proteolytic *C. botulinum*	A	1	Bhutani et al. (2005)
2002	USA	Home-made "muktuk" (from Beluga whale)	Non-proteolytic *C. botulinum*	E	12	McLaughlin et al. (2004)
2003	Germany	Home salted air-dried fish	Non-proteolytic *C. botulinum*	E	3	Eriksen et al. (2004)
2003	France	Commercial halal sausage	?[a]	B	4	Espie et al. (2003)
2004	Germany	Commercial vacuum-packed smoked salmon	Non-proteolytic *C. botulinum*	E	1	Dressler (2005)
2004	USA	Home-made pruno (2 outbreaks)	Proteolytic *C. botulinum*	A	5	Vugia et al. (2009)
2004	Italy	Restaurant preserved green olives in saline	?[a]	B	16	Cawthorne et al. (2005)
2005	UK	Travel from Georgia	Proteolytic *C. botulinum*	A	1	McLauchlin et al. (2006)
2005	Turkey	Homemade suzme (condensed) yoghurt	Proteolytic *C. botulinum*	A	10(2)	Akdeniz et al. (2007)
2005	USA	Home-salted unevicerated fish	Non-proteolytic *C. botulinum*	E	5	Sobel et al. (2007)
2005	Italy	Home canned baby food	Proteolytic *C. botulinum*	A	1(1)	Lonati et al. (2009)
2006	USA	Home fermented tofu	Proteolytic *C. botulinum*	A	2	Meyers et al. (2007)
2006	Thailand	Home canned bamboo shoots	Proteolytic *C. botulinum*	A	209	Ungchusak et al. (2007); Wongtanate et al. (2007)
2006	Canada/USA	Commercial refrigerated carrot juice	Proteolytic *C. botulinum*	A	6(1)	CDC (2006); Sheth et al. (2008)
2006	Iran	Traditional soup (Ashmast)	Non-proteolytic *C. botulinum*	E	11	Vahdani et al. (2006)
2006	Finland	Commercial vacuum-packed smoked whitefish	Non-proteolytic *C. botulinum*	E	1	Lindström et al. (2006)
2006	Taiwan	Fermented goat meat (Cinkrugan)	?[a]	B	5	Tseng et al. (2009)
2007	USA	Commercial hot dog chilli sauce	Proteolytic *C. botulinum*	A	4	CDC (2007)
2007	Australia	Commercial nacho meal	Proteolytic *C. botulinum*	A	1	Anon (2007)
2008	France	Commercial chicken enchiladas	Proteolytic *C. botulinum*	A	2	King (2008)
2009	France	Commercial vacuum-packed hot-smoked whitefish	Non-proteolytic *C. botulinum*	E	3	King et al. (2009)

[a] Not known whether proteolytic *C. botulinum* or non-proteolytic *C. botulinum*.