Chapter 12: *Clostridium botulinum*

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Potential Food Safety Hazard

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The following information on *Clostridium botulinum* is from Solomon and Lilly (1998).

C. botulinum is an anaerobic, rod-shaped sporeformer that produces a protein with characteristic neurotoxicity. Botulism, a severe food poisoning, results from ingestion of food containing botulinal toxin produced during the growth of these organisms in food. Although this food poisoning is rare, the mortality rate is high; the 962 recorded botulism outbreaks in the United States from 1899 to 1990 (CDC, 1979) involved 2320 cases and 1036 deaths. In outbreaks in which the toxin type was determined, 384 were caused by type A, 106 by type B, 105 by type E, and 3 by type F.

In two outbreaks, the foods implicated contained both types A and B toxins. The limited number of reports of C and D toxins as the causative agent of human botulism have not been generally accepted. However, all types except F and G, which have not been as thoroughly studied, are important causes of animal botulism.

Antigenic types of *C. botulinum* are identified by complete neutralization of their toxins by the homologous antitoxin; cross-neutralization by heterologous antitoxins does not occur or is minimal. There are seven recognized antigenic types: A, B, C, D, E, F, and G. Cultures of five of these types apparently produce only one type of toxin but all are given type designations corresponding to their toxin production. Types C and D cross-react with antitoxins to each other because they each produce more than one toxin and have at least one common toxin component. Type C produces predominantly C_1 toxin with lesser amounts of D and C_2, or only C_2, and type D produces predominantly type D toxin along with smaller amounts of C_1 and C_2. Mixed toxin production by a single strain of *C. botulinum* may be more common than previously realized.

There is a slight reciprocal cross-neutralization with types E and F, and recently a strain of *C. botulinum* was shown to produce a mixture of predominantly type A toxin, with a small amount of type F.

Aside from toxin type, *C. botulinum* can be differentiated into general groups on the basis of cultural, biochemical, and physiological characteristics. Cultures producing types C and D toxins are not proteolytic on coagulated egg white or meat and have a common metabolic pattern which sets them apart from the others. All cultures that produce type A toxin and some that produce B and F toxins are proteolytic. All type E strains and the remaining B and F strains are nonproteolytic, with carbohydrate metabolic patterns differing from the C and D nonproteolytic groups. Strains that produce type G toxin have not been studied in sufficient detail for effective and satisfactory characterization.

C. botulinum is widely distributed in soils and in sediments of oceans and lakes. The finding of type E in aquatic environments by many investigators correlates with cases of type E botulism that were traced to contaminated fish or other seafoods. Types A and B are most commonly encountered in foods subjected to soil contamination. In the United States, home-canned vegetables are most commonly contaminated with types A and B, but in Europe, meat products have also been important vehicles of food-borne illness caused by these types.

Optimum temperature for growth and toxin production of proteolytic strains is close to 35ºC; for nonproteolytic strains, it is 26-28ºC. Nonproteolytic types B, E, and F can produce toxin at refrigeration temperatures (3-4ºC). Toxins of the nonproteolytics do not manifest maximum potential toxicity until they are activated with trypsin; toxins of the proteolytics generally occur in fully (or close to fully) activated form. These and other differences can be important in epidemiological and laboratory considerations of botulism outbreaks. Clinical diagnosis of botulism is most effectively confirmed by identifying botulinal toxin in the blood, feces, or vomitus of the patient. Specimens must be collected before botulinal antitoxin is administered to the patient. Identifying the causative food is most important in preventing additional cases of botulism. See **Examination of Canned Foods,** Chapter 5.

Botulism in infants 6 weeks to 1 year of age was first recognized as a distinct clinical entity in 1976. This form of botulism results from growth and toxin production by *C. botulinum* within the intestinal tract of infants rather than from ingestion of preformed toxin. It is usually caused by *C. botulinum* types A or B, but a few cases have been caused by other types. Infant botulism has been diagnosed in most U.S. states and in every populated continent except Africa (Arnon, 1987).

Constipation almost always occurs in infant botulism and usually precedes characteristic signs of neuromuscular paralysis by a few days or weeks. There is a broad range of severity of illness. Some infants show only mild weakness, lethargy, and reduced feeding and do not require hospitalization. Many have shown more severe symptoms such as weakened suck, swallowing, and cry; generalized muscle weakness; and diminished gag reflex with a pooling of oral secretions. Generalized muscle weakness and loss of head control in some infants reaches such a degree of severity that the patient appears "floppy." In some hospitalized cases, respiratory arrest has occurred, but most were successfully resuscitated, and with intense supportive care have

ultimately recovered. As a result, the case-fatality rate (2%) for this form of botulism is low. Recovery usually requires at least several weeks of hospitalization (Arnon, 1987).

Honey, a known source of *C. botulinum* spores, has been implicated in some cases of infant botulism. In studies of honey, up to 13% of the test samples contained low numbers of *C. botulinum* spores (Hauschild et al., 1988). For this reason, the FDA, the Centers for Disease Control and Prevention (CDC), and the American Academy of Pediatrics recommend not feeding honey to infants under the age of 1 year.

Control Measures

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Measures to prevent botulism include reduction of the microbial contamination level, acidification, reduction of moisture level, and whenever possible, destruction of all botulinal spores in the food. Heat processing is the most common method of destruction. Properly processed canned foods will not contain viable *C. botulinum*. Home-canned foods are more often a source of botulism than are commercially canned foods, which probably reflects the commercial canners' great awareness and better control of the required heat treatment.

A food may contain viable *C. botulinum* and still not be capable of causing botulism. If the organisms do not grow, no toxin is produced. Although many foods satisfy the nutritional requirements for the growth of *C. botulinum*, not all of them provide the necessary anaerobic conditions. Both nutritional and anaerobic requirements are supplied by many canned foods and by various meat and fish products. Growth in otherwise suitable foods can be prevented if the product, naturally or by design, is acidic (of low pH), has low water activity, a high concentration of NaCl, an inhibitory concentration of $NaNO_2$ or other preservative, or two or more of these conditions in combination. Refrigeration will not prevent growth and toxin formation by nonproteolytic strains unless the temperature is precisely controlled and kept below 3ºC. Foods processed to prevent spoilage but not usually refrigerated are the most common vehicles of botulism (Solomon and Lilly, 1998).

FDA Guidelines

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FDA Guidelines for *C. botulinum* in fish and fishery products.

Growth

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Limiting conditions for C. *botulinum* growth.

Heat Resistance

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Spore heat resistance of *C. botulinum* nonproteolytic type E.

Temp.		D-Value	Strain	Medium	Reference
(ºC)	(ºF)	(min.)			
73.89	165	12.97	Beluga	Crabmeat	Lynt et al., 1977
73.89	165	10.39	Alaska	Crabmeat	Lynt et al., 1977
73.89	165	6.80	G21-5	Crabmeat	Lynt et al., 1977
73.9	165	8.66	Mixed	Surimi	Rhodehamel et al., 1991
76.67	170	4.07	Beluga	Crabmeat	Lynt et al., 1977
76.67	170	3.04	Alaska	Crabmeat	Lynt et al., 1977
76.67	170	2.38	G21-5	Crabmeat	Lynt et al., 1977
76.7	170	3.49	Mixed	Surimi	Rhodehamel et al., 1991
79.4	175	2.15	Mixed	Surimi	Rhodehamel et al., 1991
79.44	175	1.65	Beluga	Crabmeat	Lynt et al., 1977
79.44	175	1.35	Alaska	Crabmeat	Lynt et al., 1977
79.44	175	1.10	G21-5	Crabmeat	Lynt et al., 1977
82.2	180	1.22	Mixed	Surimi	Rhodehamel et al., 1991
82.22	180	0.74	Beluga	Crabmeat	Lynt et al., 1977
82.22	180	0.51	Alaska	Crabmeat	Lynt et al., 1977
82.22	180	0.63	G21-5	Crabmeat	Lynt et al., 1977
82.22	180	0.62	25V-1	Crabmeat	Lynt et al., 1977
82.22	180	0.49	25V-2	Crabmeat	Lynt et al., 1977
85.00	185	0.29	Beluga	Crabmeat	Lynt et al., 1977

Spore heat resistance of *C. botulinum* type B.

Temp.		D-Value	Strain	Medium	Reference
(ºC)	(ºF)	(min.)			
88.9	192	12.9	N/S	Crabmeat	Peterson et al., 1997
89.5	193	11.1	N/S	Crabmeat	Peterson et al., 1997
90.0	194	9.5	N/S	Crabmeat	Peterson et al., 1997

90.6	195	8.2	N/S	Crabmeat	Peterson et al., 1997
91.1	196	7.1	N/S	Crabmeat	Peterson et al., 1997
91.7	197	6.1	N/S	Crabmeat	Peterson et al., 1997
92.2	198	5.3	N/S	Crabmeat	Peterson et al., 1997
92.8	199	4.5	N/S	Crabmeat	Peterson et al., 1997
93.4	200	3.9	N/S	Crabmeat	Peterson et al., 1997
93.9	201	3.4	N/S	Crabmeat	Peterson et al., 1997
94.5	202	2.9	N/S	Crabmeat	Peterson et al., 1997

N/S = Not specified

Incidence in Fish and Fishery Products

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Dungeness crab

Occurrence of *C. botulinum* in the intestinal tract, gills, and shell of Dungeness crab (*Cancer magister*) crab from the Pacific Coast of the United States (Eklund and Poysky, 1967).

Area	No. crab	% crab positive	*C. botulinum* type
Ketchikan, AK	21	57	E
Bellingham, WA	18	61	B, E
	6	66	E
	32	75	E
Gray's Harbor, WA	40	75	A, E
Columbia River	20	87	E

Eureka, CA	50	14	E
Fort Bragg, CA	43	30	A, B, E
San Francisco, CA	50	12	B, C, E

Great Britain fishing grounds

Distribution of *C. botulinum* type E in the marine environment of Great Britain (Cann et al., 1967).

Source	Sample	No. tested	No. containing *C. botulinum* type E
Shops in Britain	Vacuum packed fish	646	5
Skagerrak	White fish intestines	130	0
Skagerrak	Whole white fish	130	0
North Sea	White fish intestines	96	0
North Sea	Herring intestines	200	0
Norwegian Sea	Herring intestines	22	3
Norwegian Sea	Whole herring	44	24
England & Wales	Shellfish	106	0

Gulf of Maine fish

Total numbers of composite fish intestine (10-1/2 intestines per sample) assayed and the number containing *C. botulinum* toxin (Nickerson et al., 1967).

Species	Number of composite intestine samples	Number of samples containing type E toxin
Haddock	116	6
Cod	41	1
Flounder, black back	45	4

Flounder, dab	31	1[1]
Pollock	9	0

[1]Non-specific

Toxin tests on Most Probable Number (MPN) cultures of frozen intestines corresponding to samples previously found to contain type E *C. botulinum* (Nickerson et al., 1967).

Sample	Sample number	MPN Type E cells per 100g
Haddock	1	10
	2	<4
	3	4
	4	4
	5	<4
	6	<4
Cod	1	<4
Black back	1	<4
	2	4
	3	<4
	4	<4

Menhaden surimi

Seven of 565 (1.2%) test portions (250g) of menhaden surimi without added cryoprotectants contained *C. botulinum* type E spores (Rhodehamel et al., 1991).

Seafood cocktails

C. botulinum type E was not detected in 35 samples of commercial seafood cocktails, ranging in pH from 4.10 to 4.85 (Lerke, 1973).

Smoked eel

Two of 10 (20%) different samples of smoked eel tested positive for *C. botulinum* type E (Abrahamsson, 1967).

Smoked salmon

119 samples of smoked salmon in Denmark were examined for *C. botulinum* spores. *C. botulinum* type B was isolated from 2 samples (1.68%). A strong inhibitory effect of formaldehyde against *C. botulinum* in smoked salmon was noted (Nielsen and Pedersen, 1967).

U.S. Pacific coast fish

C. botulinum toxicity screening results from fish gills and viscera (Craig and Pilcher, 1967).

Fish	No. of samples tested	No. of samples toxic	% toxic	No. of samples typed as type E
Sockeye salmon	56	12	21.4	4
Chinook salmon	91	15	16.5	4
Silver salmon	210	21	10.0	10
Sturgeon	27	3	17.0	3
Steelhead	36	7	19.4	5, 2B
Bottom fish	113	19	16.8	13, 1A

Comparative incidence of toxic specimens in ocean-caught and river caught salmon (Craig and Pilcher, 1967).

Species/location	Samples tested	No. toxic samples	% toxic samples
Silver salmon			
Port Angeles	65	1	1.5
La Push	50	3	6.0
Westport	43	4	9.3
Columbia River	28	9	32.3
Newport-Depot Bay	34	1	2.9
Chinook salmon			
Columbia River	74	15	20.3
Westport Newport	75	5	6.7

Comparative incidence of toxic specimens in bottom fish caught near Columbia River and in a distant coastal location (Craig and Pilcher, 1967).

Location	No. tested	No. toxic	% toxic
Coos Bay	54	5	9.2
Astoria	59	14	23.7

Comparative incidence of toxic specimens in miscellaneous samples tested (Craig and Pilcher, 1967).

Sample	No. tested	No. toxic	% toxic
Clams	8	1	12.5
Oysters	3	0	0
Smelt	2	0	0
Smoked fish	15	2	7.5

U.S. Retail fish

Occurrence of *C. botulinum* in random samples[a] of fish and other seafoods obtained from retail markets, wholesale distributors/processors, a fish farm, and fish hatchery, between 1984-1985 (Baker et al., 1990).

Common name	Origin	No. pos./ No. tested	%	Types
Butterfish	California Coast	0/5	0	NA
Cat fish, farm raised	Modesto, CA	3/4	75.0	3A, 1F
	Indianola, MS	1/5	20.2	1A
Halibut	Pacific Coast	3/11	27.3	3A
King fish	Unknown	0/5	0	NA
Ling cod	Pacific Coast	1/5	20.0	1A
Mackerel	Unknown	0/5	0	NA

Ocean perch	Pacific Coast	2/7	28.6	2A
Pacific oyster	Pacific Coast	0/5	0	NA
Prawns	Unknown	0/5	0	NA
Rockfish, G.I. tract		3/10	30.0	3A
Rockfish, composite[a]		6/6	100.0	3A
Rockfish, retail market fillets		0/8	0	NA
Rex sole	California coast	2/9	22.0	1A, 1B
King salmon gills	Nimbus River, CA	2/18	11/1	2A
King salmon G.I. tract	Nimbus River, CA	3/8	37.5	3A
King salmon composite[b]	Nimbus River, CA	3/3	100.0	3A
King salmon retail market fillet	Alaska	2/14	14.3	2A, 1B
Sardines	Canada	2/5	40.0	1A, 1B
Scallops	East Coast	1/6	16.6	1A
Shark	Unknown	0/1	0	NA
Shrimp	Unknown	0/5	0	NA
Smelt	Unknown	0/5	0	NA
Squid	Unknown	0/5	0	NA
Trout	Unknown	2/6	33.3	1A, 1B

[a]Sample units were 70g of muscle homogenate
[b]Composite sample: Homogenized flesh from 4-9 fish.
NA = Not applicable.

Analytical Procedures

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Food sampling and preparation of sample homogenate (USFDA) Top

Clostridium botulinum (USFDA) Top

Other analytical procedures Top

- *Clostridium botulinum* and its toxins in foods (AOAC, 1995)

Commercial Test Products

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Commercial test products for *C. botulinum*.

Test Kit	Analytical Technique	Approx. Total Test Time[1]	Supplier
Probelia PCR System	Polymerase chain reaction	30 h	BioControl Systems, Inc. Contact: Robin Forgey 12822 SE 32nd St. Bellevue, WA 98005 Phone: 425/603-1123 E-mail: info@rapidmethods.com Web: www.rapidmethods.com

[1]Includes enrichment

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