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Botulism Due to *Clostridium baratii* Type F Toxin

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Botulism results from consumption of preformed toxin or in vivo toxin elaboration in wounds or intestine. Of U.S. food-borne botulism cases since 1950, the majority were due to toxin A, but a significant number of suspect cases were never confirmed by culture or toxin detection. We report here a possible case of food-borne botulism attributed to toxin F production by a *Clostridium baratii* organism isolated from food consumed by the patient. The isolation of a toxin-producing *Clostridium* species other than *Clostridium botulinum* from food and stool requires deviation from the usual laboratory protocols, which may account for the lack of complete laboratory confirmation of clinically diagnosed cases.

Botulism is caused by a neurotoxin produced by a member of the genus *Clostridium*, usually *Clostridium botulinum.* Organisms classified in this species produce neurotoxins distinguished by their serologic properties into toxin types A, B, C, D, E, and F. Former *C. botulinum* toxin G-producing organisms are now classified in a new species, *C. argentinense* (1). In the three most common forms of botulism (food-borne, wound, and infant), disease is predominantly caused by C. *botulinum* toxin A, although toxins B and E are also important (12). Botulism is so rarely caused by other members of the genus that a literature search revealed only four published papers that linked human disease to two other species, *Clostridium baratii* and *Clostridium butyricum. C. butyricum* isolated from two cases of infant botulism was demonstrated to produce type E toxin (2); *C. baratii* isolated from an adult and two infant botulism cases was shown to produce type F toxin (6, 10, 11). In these three cases of reported *C. baratii* botulism, the disease was thought to be due to intestinal colonization by the organism, not food-borne organisms. We report here the clinical and laboratory details of a case of *C. baratii* type F toxin botulism in an adult where the toxin-producing organism was isolated from a food the patient had consumed.

Case report. A 41-year-old woman was transported by ambulance to the hospital in January 2001. Her complaint was shortness of breath and weakness subsequent to an upper respiratory infection. In addition, however, she stated that she had vomited several times that morning and felt dizzy when she moved her head. She had consulted her personal physician 2 days previously because of the upper respiratory infection, and he had prescribed amoxicillin.

Routine admission studies showed no abnormalities. The decision was made to transfer her to her usual hospital for further evaluation. While lying on the gurney waiting for transfer, she suddenly developed bradycardia and went into respiratory arrest. Resuscitation was done immediately. Although she was able to move her extremities in response to pain and verbal commands, all extremities showed weakness and her pupils were now dilated and less reactive to light. She was admitted to the intensive care unit where subsequent testing was undertaken to determine the explanation for her collapse. Tomography of her head was normal; pulmonary circulation was normal. However, the patient could not breathe without ventilator support.

Several consultant physicians examined the patient within 24 h of admission. They each noted that the patient had no difficulty moving all extremities, extraocular muscles were intact, and pupils were reactive to light. Because of onset of fever, broad-spectrum antibiotics were started by the infectious disease consultant.

About 30 h after admission, nurses noted decreased voluntary movements. The patient was awake but unable to open her eyes. A neurological consultation showed normal strength in both hands and feet but no proximal movement, and muscle tone was flaccid. Deep tendon reflexes were absent. She was able to make facial grimaces and move her tongue slightly, but her pupils were fixed. The diagnosis of botulism was considered, but an extensive search for a possible abscess causing wound botulism was negative.

Over the next 12 h, however, the patient's condition deteriorated and voluntary muscle activity was lost. One unit of bivalent A/B botulinum antitoxin was given 60 h after admission. When pretreatment serum testing suggested the presence of type E toxin, A/B/E antitoxin was administered 3 days later. Subsequent toxin testing in our laboratory confirmed the presence of type F toxin in the pretreatment serum, but no additional specific antitoxin was administered to the patient.

By day 7 after admission, return of motor activity was noted in the patient. Distal, proximal, and upper and lower extremity movements gradually returned; deep tendon reflexes were normal by day 21. She was transferred to a long-term care facility at 7 weeks and was ventilator dependent for 12 weeks.

The only food known to be consumed solely by the patient was commercially canned tuna; two small cans were eaten about 18 h prior to first symptoms. The family recovered these cans from the garbage and submitted them to the laboratory along with an unopened can of the same lot number. In addition, a plastic storage bag containing spaghetti with a tomato meat sauce was also submitted by the family for analysis.

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TABLE 1. Toxicological and microbiological findings

Specimen source	Day after onset	Presence of preformed toxin[a]	Toxin type identified[b]	Toxigenic organism isolated
Serum	2	+	F[c]	
Stool	3	−		Nontoxigenic *C. baratii*
Serum	6	−		
Peritoneal fluid	8	−		
Tuna can A		−		
Tuna can B		+	F	*C. baratii*[d]
Spaghetti noodles and meat sauce		NT	F	*C. baratii*[e]

[a] +, toxin detected; −, no toxin detected; NT, not tested.
[b] The toxin type identified was either preformed or produced by isolate.
[c] The neurotoxin was also neutralized by type E antitoxin, but with lower efficiency.
[d] Of six isolates identified and tested, all produced type F toxin and three required trypsin for detection.
[e] Of eight isolates recovered from enrichment culture only, 7 isolates were tested for toxin production; all produced type F toxin, and none required trypsin.

Laboratory methods. Initial serum and stool specimens obtained prior to antitoxin administration, sera taken on the fifth and ninth days after admission (3 days after bivalent A/B antitoxin administration and 2 days after trivalent A/B/E antitoxin administration), and the contents of the tuna cans were examined for botulinal neurotoxin by mouse bioassay (3, 4). In addition, the fecal specimen, tuna cans, and spaghetti with tomato meat sauce were cultured by standard methods for clostridial organisms (3). All potentially toxigenic organisms identified were also tested for toxin by mouse bioassay.

Laboratory findings. The laboratory findings are summarized in Table 1. The first serum specimen was rapidly lethal for mice; the unneutralized pair died within 18 h. The lethality was partially neutralized by monovalent antitoxin E and completely neutralized by monovalent antitoxin F. There was no neutralization with type A or type B monovalent antitoxin. Subsequent serum specimens taken after administration of the two types of antitoxin (A/B, A/B/E) were not toxic for mice.

Of the two tuna cans retrieved from the garbage, only one demonstrated toxin activity in the rinse fluid and contents. This can (B), prior to rinsing with gelatin phosphate, had no discernible residue of tuna but contained a mixture of grease, spaghetti, and meat. The other can (A) contained approximately 2 g of residual tuna, but the rinse fluid and contents were not toxic for mice. Culture of both cans yielded isolates with biochemical characteristics of *C. baratii* from can B but no *Clostridium* sp. from can A. Both the rinse fluid and the culture fluid of the presumptive *C. baratii* isolates from can B were lethal to mice, and the activity was neutralized by monovalent antitoxin F.

Culture of the spaghetti and sauce mixture transported in a plastic storage bag yielded several colonies of presumptive *C. baratii.* The spaghetti and sauce mixture was not tested for preformed toxin because of elapsed storage time. The culture fluid of all isolates was lethal for mice except when neutralized by antitoxin F.

Neither the extract nor the enrichment supernatant of the fecal specimen (obtained at the same time as the initial serum specimen) was toxic for mice. Culture of the fecal specimen did grow a single colony of presumptive *C. baratii*, but the culture fluid from this isolate was nontoxigenic in the mouse bioassay.

The biochemical characteristics of isolates differed slightly, but all yielded a biochemical profile consistent with *Clostridium baratii.* The typical biochemical profiles of the isolates are listed in Table 2. Subsequent testing by Sydney Finegold and staff of the Anaerobic Bacteriology Research Laboratory at the Wadsworth Veterans Administration Hospital confirmed the fecal and neurotoxin-producing food isolates to be *C. baratii.* The confirmations were done using whole-cell long-chain fatty acid methyl ester analysis.

Discussion. The diagnosis of either food-borne botulism or adult colonization (intestinal) botulism in the patient reported here was substantiated by the detection of toxin in serum and toxin-producing organisms in the food consumed. Specific diagnosis as to the type of botulism disease suffered by this patient was confounded by the fact that other individuals in the household ate the spaghetti and sauce, yet they contracted no obvious disease. Additionally, since the patient had recently received antibiotic treatment for her respiratory illness (amoxicillin), this might have predisposed her to adult infectious botulism by altering her normal intestinal flora; at most, however, she took 2 days of treatment. At the time of laboratory testing, toxin F-producing *C. baratii* was found in the spaghetti and sauce mixture and preformed toxin F was demonstrated in the presumed residue of the spaghetti sauce meat in tuna can B. Whether this is an accurate reflection of the status of the

TABLE 2. Characteristics of *C. baratii* isolates

Characteristic	Result for *C. baratii* from:	
	Food specimens[a]	Stool specimen
Reactions on egg yolk agar		
Lipase	−	−
Lecithinase	+	+
Substrate[b]		
Indole	−	−
N-acetylglucosamine	+	+
Alpha-glucosidase	−	−
Alpha-arabinosidase	−	−
Beta-glucosidase	+	+
Alpha-fucosidase	−	−
Phosphatase	−	−
Alpha-galactosidase	+	+
Beta-galactosidase	−	+
C2 esterase	−	−
Arginine dihydrolase	+	+
Leucine aminopeptidase	−	−
Proline aminopeptidase	−	−
Pyroglutamic acid arylamidose	+	+
Tyrosine aminopeptidase	−	−
Arginine aminopeptidase	+	+
Alanine aminopeptidase	−	−
Histidine aminopeptidase	−	−
Phenylalanine aminopeptidase	−	−
Glycine aminopeptidase	−	−
Catalase	−	−
Type of neurotoxin	F	None detected

[a] Includes isolates from both tuna can B and spaghetti with meat sauce mixture.
[b] API AN-IDENT SYSTEM (Biomerieux).

meat sauce at the time of consumption by members of the household is unknown.

The extremely rapid lethal effect of the toxin in mice is juxtaposed against the relatively rapid recovery of the patient. This difference in the apparent effect of a botulinal neurotoxin has not been noted in Los Angeles County prior to this case. It may be the reflection of a unique property of the *C. baratii* toxin F as opposed to the other *C. botulinum* toxins, including toxin F.

Recently, there have been several studies on the genetic and structural relationships among clostridial neurotoxins (5, 8, 9, 13). Sequence analysis has shown that there is high genetic homology between the toxins E produced by *C. butyricum* and *C. botulinum* but low relatedness between the genes coding for the toxins F of *C. baratii* and saccharolytic *C. botulinum.* This suggests that some strains of *C. baratii* possess the capacity to synthesize a unique type of toxin F, whereas *C. butyricum* acquires its ability to produce toxin E through lateral gene transfer from *C. botulinum* (8, 13).

Furthermore, the light chain of *C. baratii* toxin F shows sequence identities of 64.2 and 55.2% with the light chains of *C. botulinum* toxins F and E, respectively. The heavy chain of *C. baratii* toxin F shows higher sequence similarity, 73.6 and 68.4%, with the heavy chains of *C. botulinum* toxins F and E, respectively (5). Since the heavy chains apparently determine the receptor function, the noted similar homologies between the heavy chains of F and E indicate a shared receptor. These facts may explain the apparent neutralization of *C. baratii* toxin F by both *C. botulinum* antitoxins E and F while confirming the partial sequence uniqueness of the *C. baratii* toxin F. This structural difference is further supported by studies showing that it requires more type F botulinal antitoxin to neutralize a given amount of *C. baratii* toxin F than it does to neutralize botulinal toxin F and that *C. baratii* toxin F is more susceptible to cross-neutralization by type E antitoxin (7). The partial sequence uniqueness of *C. baratii* toxin F is in contrast to the sequence identities found between the light and heavy chains of *C. butyricum* toxin E and those of *C. botulinum* toxin E, 96 and 98.1% homology, respectively (13).

While botulism due to *C. baratii* is rarely reported, it may be more common and missed because of the protocols followed by most laboratories for screening. In March 1986 this laboratory detected toxin F in a stool specimen from a patient, but despite repeated attempts, no organism was isolated. At that time, there was not recognition of the possible importance of *C. baratii* and the laboratory was looking exclusively for *C. botulinum.* Usually, isolation media used for screening contain egg yolk as a differential ingredient to detect the lipase-positive colonies of *C. botulinum. C. baratii* is lipase negative and lecithinase positive, a profile not shared by any recognized group of *C. botulinum*. In the present case, a large number of colonies were picked for every isolation attempt; these were identified, and toxin testing was done on selected isolates identified as possible *C. baratii* or other similar clostridia. This was a very labor-intensive undertaking from the food and stool specimens, especially since the morphological characteristics of *C. baratii* are unfamiliar to most microbiologists. In fact, the latest edition of the *Manual of Clinical Microbiology* (1) gives only brief mention of *C. baratii*, does not describe its morphology, and omits it from the table of differential characteristics of clostridia.

Of U.S. food-borne botulism outbreaks since 1950, only 67% were identified as to toxin type, and of 309 persons diagnosed clinically with botulism between 1975 and 1988, only 65% had either a positive culture or a positive toxin test (3). Some of the suspect botulism cases that are culture and toxin negative may be true cases missed because laboratories are not screening for clostridia other than *C. botulinum*.

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