Intestinal Toxemia Botulism in Two Young People, Caused by \textit{Clostridium butyricum} Type E

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Two unconnected cases of type E botulism involving a 19-year-old woman and a 9-year-old child are described. The hospital courses of their illness were similar and included initial acute abdominal pain accompanied by progressive neurological impairment. Both patients were suspected of having appendicitis and underwent laparotomy, during which voluminous Meckel's diverticula were resected. Unusual neurotoxigenic \textit{Clostridium butyricum} strains that produced botulinum-like toxin type E were isolated from the feces of the patients. These isolates were genotypically and phenotypically identical to other neurotoxigenic \textit{C. butyricum} strains discovered in Italy in 1985–1986. No cytotoxic activity of the strains that might explain the associated gastrointestinal symptoms was demonstrated. The clinical picture of the illness and the persistence of neurotoxigenic clostridia in the feces of these patients suggested a colonization of the large intestine, with in vivo toxin production. The possibility that Meckel's diverticulum may predispose to intestinal toxemia botulism may warrant further investigation.

Botulism in humans is a serious paralytic illness caused by neurotoxins that are usually produced by \textit{Clostridium botulinum} types A, B, and E. Rare cases have been caused by 2 other species of clostridia, \textit{Clostridium baratii} and \textit{Clostridium butyricum}, which produce, respectively, type F and type E botulinum–like neurotoxins [1–5].

The resulting illness may affect all ages from newborns to adults (table 1). In addition to the classic form caused by the ingestion of preserved foods contaminated with botulinum toxin (foodborne botulism) and a rare infectious form affecting traumatized tissue (wound botulism), 2 new forms of botulism have been identified in the last 2 decades: one affects only infants in their first year of life (infant botulism) [6] and the other affects older children and adults (infant-like botulism) [7]. Both forms result from the colonization of the intestinal tract by neurotoxigenic clostridia.

Thus, the term botulism is used to indicate not only the classic food poisoning but also the other clinical forms that result from colonization of the intestinal tract or from tissue infection with in vivo production of the toxin. Because both the infant and adult infectious intestinal forms occasionally involve clostridia other than \textit{C. botulinum}, a new descriptive term, intestinal toxemia botulism, has been proposed for the infectious intestinal forms to distinguish them from the other 2 forms [8].

In Italy, although foodborne botulism [9], infant botulism [2], and wound botulism [10] have been described, cases of infant-like botulism in older children or adults have not thus far been reported. This last form of botulism has been well documented [7] and prevalently reported in the United States [8], where it has been described in a limited number of cases, almost all of which were associated with gut flora and pH altered by surgery and/or drugs.

Herein we describe the clinical and microbiological findings from 2 unconnected cases of type E botulism, 1 in an adult and 1 in an older child, which we suspect resulted from intestinal colonization. The 2 cases occurred 1 year apart in different provinces of northern Italy and were associated with similar illnesses. In both cases, neurotoxigenic \textit{C. butyricum} was isolated from fecal material.

The phenotypic characteristics of the 2 strains (Istituto Superiore di Sanità [ISS] CL 86 and CL 109), antibiotic resistance, sugar fermentation patterns, and other biochemical profiles were compared. Likewise, the genotypic characteristics of the 2 strains were investigated and compared with those of other \textit{C. butyricum} strains (ISS CL 20 and CL 21) that produce type
E botulinum–like toxin, which were isolated from 2 cases of infant botulism that occurred in Italy 10 years earlier [2].

Finally, to investigate a possible relationship between the gastrointestinal illness observed in both patients and the neurotoxigenic C. butyricum strains isolated, we studied the possible cytotoxic effect of these clostridial culture supernatants on cell cultures [11].

Case Reports

Case 1. A 9-year-old boy was hospitalized in Lugo di Romagna, near Ferrara in northern Italy, on 6 December 1994 because of acute abdominal pain and vomiting. Although afebrile, he was diagnosed initially with appendicitis. On day 3 after admission, although he was alert and fully oriented, his examination was notable for worsening diplopia, bilateral mydriasis, dysphonia, dry mouth, dry eyes, constipation, tympanic abdomen, urinary retention, tachycardia, tachypnea, and dyspnea.

His worsening condition prompted transfer for intensive care at another hospital, where he immediately underwent laparotomy. He was found to have abundant ascites and a large, inflamed Meckel’s diverticulum, which was resected along with his appendix. Neither the diverticulum nor the ascites were retained for laboratory analysis. His intestinal loops were distended by gas and liquid feces. He was treated with ceftriaxone (1 g/day) and clavulanic acid (1 g/day) for 15 days after surgery.

On day 5, he developed paralysis of the facial muscles and upper limbs and displayed sensory disturbances and dysautonomia. Because of progressive respiratory failure, he was placed on mechanical ventilation for 3 days. Electromyography displayed normal nerve conduction and facial nerve action potential amplitude. Although his physicians suspected either polyradiculoneuritis with dysautonomia or autoimmune disease of the CNS as the cause of weakness, botulism was also considered in the differential diagnosis.

His routine blood tests were normal, and stool culture did not detect Salmonella, Shigella, or Campylobacter species, Ver- sinia enterocolitica, adenovirus, or rotavirus. No antibodies to CNS antigens were found. Serum obtained at admission and a rectal swab collected on hospital day 5 contained no detectable botulinum toxin or neurotoxigenic clostridia.

Regular bowel movements resumed after intestinal cannulation on day 13. His general condition then improved rapidly, although mydriasis, dysphonia, and dryness of the mouth and eyes persisted. C. butyricum that produced type E botulinum–like toxin was isolated from a stool specimen collected on day 16 (this isolate was not received at the national botulism laboratory until after his second hospital discharge). He was discharged for the first time in good condition on day 25 with a diagnosis of acute abdomen and radiculopathy.

One week later, he was again hospitalized for acute abdominal pain, bloody feces, vomiting, and fever. The hemorrhage worsened the following day and persisted for 5 days. Therapy included rehydration and administration of rifampicin at 1 g/day for 7 days. Routine microbiological analyses of the feces were negative. His abdominal echogram and radiographs showed no abnormalities. He was discharged after 5 days of hospitalization, on 12 January 1995, with a tentative diagnosis of acute infectious or allergic enterocolitis.

Case 2. On 28 December 1995, a 19-year-old woman was hospitalized in Rovigo (Veneto Province), in northern Italy, with acute abdominal pain, nausea, vomiting, diplopia, dysphagia, and constipation. She was alert and afebrile but had ptosis and mydriasis. Her symptoms began 2 days after she had returned from her Christmas holidays. She had traveled and shared all meals with a friend who remained healthy.

Appendicitis was suspected, and a laparotomy was immediately done. Her surgeons found abundant ascites, distended intestinal loops, and intussusception of an inverted, inflamed Meckel’s diverticulum that they resected. Neither the diverticulum nor the ascites were submitted for further analyses. She was treated with cefazidime at 3 g/day for 15 days.

After the laparotomy, her paralysis rapidly progressed to a point at which she appeared comatose with fixed pupils. On day 2 she was transferred for intensive care to Padua, where she received mechanical ventilation for 4 days. A cranial CT appeared normal. Her electromyogram showed a presynaptic
neuromuscular block compatible with botulism. Trivalent ABE botulinum antitoxin (Behring, Marburg, Germany) was administered. The clinical diagnosis of botulism was confirmed after \textit{C. butyricum}–producing type E–like botulinum toxin was isolated from a rectal swab taken on day 6.

By day 7, the patient had resumed bowel movements and oral feedings, although she was plagued by recurrent abdominal pain, vomiting, and alternating constipation and diarrhea. By day 12, her only remaining symptom was an ocular nerve disturbance. By day 23, she had completely recovered. She was discharged with a diagnosis of foodborne botulism of unknown origin.

**Laboratory Analyses**

*Botulinum toxins and spore research.* The serum and fecal samples from the patients were examined for botulinum neurotoxins by mouse bioassay [12]. Samples of feces and fecal swabs were cultured for neurotoxigenic clostridia by standard methods [12]. Spore count of toxigenic organisms was done by the most probable number method [13]. To facilitate isolation of the neurotoxin-producing organisms, the lipase-positive and lipase-negative colonies from egg yolk agar plates were screened for botulinum toxin genes by PCR assay [14] and then for the production of neurotoxins by mouse bioassay.

*Identification and characterization of isolates.* Strains from toxigenic colonies were identified by cultural, physiological, and serological characteristics [15, 16]. To identify differences between the toxigenic \textit{C. butyricum} strains that were isolated from these 2 patients (ISS CL 86 and CL 109) and the other 2 strains of toxigenic \textit{C. butyricum} isolated 10 years earlier (ISS CL 20 and CL 21), the antibiotic susceptibility of all 4 strains was compared with that of 2 nontoxigenic strains of \textit{C. butyricum} (ISS CL 19 and ATCC [American Type Culture Collection] 19398). Pulsed-field gel electrophoresis (PFGE) analysis was used to compare the 4 strains with \textit{C. butyricum} strain ATCC 19398.

*PFGE analysis.* Clostridial strains were grown and embedded in low-melting-point agarose plugs to obtain high-molecular-weight DNA as described by Lin and Johnson [17]. Restriction with the rare-cut endonucleases \textit{SmaI}, \textit{KspI}, \textit{XhoI}, \textit{NruI}, and \textit{MluI} (Boehringer Mannheim, Mannheim, Germany) was allowed to occur overnight at the conditions suggested by the manufacturer. DNA fragments were then separated by a contour-clamped homogeneous electric field electrophoresis apparatus (CHEF-DRII; Bio-Rad Laboratories, Hercules, CA) [18].

*Susceptibility to antibiotics.* The disk diffusion assay with criteria for determining the antibiotic susceptibility of \textit{C. butyricum}, described by Magot [19], was used to establish the susceptibility of the neurotoxigenic organisms isolated from the patients. Commercially available disks (Oxoid, Basingstoke, UK) soaked in the following antibiotics were used: penicillin (6 μg), tetracycline (30 IU), chloramphenicol (30 μg), erythromycin (15 IU), metronidazole (5 μg), and clindamycin (2 μg).

*Cytotoxic activity.* The possibility that the neurotoxigenic strains of \textit{C. butyricum} isolated from the 2 patients might also be responsible for the enterocolitis was considered. To test this hypothesis, culture filtrate neutralized with type E anti-botulinum serum was inoculated on a Vero epithelial cell culture and incubated according to Popoff et al. [11]. Presence of cytopathic activity in 50% of the cell culture was considered a positive result. The 2 neurotoxigenic \textit{C. butyricum} strains previously recovered from 2 infant botulism cases (CL 20 and CL 21) were also assayed.

*Food analysis.* Food samples taken from the home of patient 1 included homemade sausage from the same batch consumed by the patient and his parents. The sausage was analyzed for toxins and for toxigenic clostridia by the methods described above. It was not possible to identify the pubs and inns where patient 2 had eaten during her Christmas holidays.

*Antitoxin antibody studies.* Possible serum neutralizing (antitoxin) antibodies were sought by the mouse bioassay according to the method of Griffin et al. [20], who recently demonstrated an active immune response to botulinum toxin type A in a patient with adult intestinal (or possibly wound) botulism. Only the serum of patient 1, taken 1 year after hospital discharge, was available for this assay.

**Results**

The laboratory findings from the clinical samples of the 2 patients are summarized in table 2. The serum sample obtained from patient 2 was not examined because it was drawn after treatment with botulinum antitoxin.

Both clostridial strains isolated from enrichment cultures from the 2 patients (ISS CL 86 and CL 109) displayed the characteristics of \textit{C. butyricum} listed in table 3. However, the 2 strains differed from type-strain \textit{C. butyricum} in their capacity to produce a toxin neutralized by type E botulinum antitoxin, because of the presence of the type E toxin gene in their genome.

Table 4 shows the results of the antibiotic susceptibility

<table>
<thead>
<tr>
<th>Patient, sample</th>
<th>Days after admission</th>
<th>Presence of toxin</th>
<th>Toxigenic organism isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Feces (swab)</td>
<td>5</td>
<td>NAa</td>
<td>–</td>
</tr>
<tr>
<td>Feces</td>
<td>16</td>
<td>–</td>
<td>\textit{Clostridium butyricum}</td>
</tr>
<tr>
<td>Patient 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>6</td>
<td>NA</td>
<td>\textit{C. butyricum}</td>
</tr>
<tr>
<td>Feces (swab)</td>
<td>6</td>
<td>NAa</td>
<td>\textit{C. butyricum}b</td>
</tr>
<tr>
<td>Feces</td>
<td>9</td>
<td>–</td>
<td>\textit{C. butyricum}b</td>
</tr>
<tr>
<td>Feces</td>
<td>11</td>
<td>–</td>
<td>\textit{C. butyricum}b</td>
</tr>
</tbody>
</table>

*NOTE.* NA, not analyzed; –, negative.

\(^a\) Insufficient sample size.

\(^b\) \(1 \times 10^6\) spores/g.
testing for both isolates compared with the antibiotic susceptibilities of the 2 previously isolated strains (ISS CL 20 and CL 21) and 2 nontoxigenic C. butyricum strains isolated from food samples (ISS CL 19 and ATCC 19398). PFGE analysis showed no differences among the 4 toxigenic strains of C. butyricum (figure 1); however, their banding profiles were completely different from that of the nontoxigenic C. butyricum ATCC 19398. Finally, no cytotoxic effect was exhibited by the 4 toxigenic strains.

The microbiological and toxicological analysis of food samples taken from the home of patient 1, including the homemade sausages originating from the same batch as those consumed by the patient and his parents, revealed the presence of neither preformed botulinum or type E botulinum-like toxin nor spores of an organism that could produce type E toxins.

No serum neutralizing (antitoxin) antibody was detected. This negative result might have occurred because type E neurotoxin appears to be a poor inducer of neutralizing (antitoxin) antibody in foodborne botulism [21].

Discussion

We report 2 unconnected cases of botulism in young people that were confirmed by the identification of neurotoxigenic type E C. butyricum in their feces and that probably resulted from an intestinal colonization with this organism. In both cases, the illness presented as acute abdominal pain suspected to be appendicitis. During appendectomy, each patient was found to have a Meckel’s diverticulum, abundant ascites, and intestinal loops distended by accumulated liquid and gas. The gastro-intestinal signs were accompanied by neurological symptoms and signs that evolved after surgical intervention until they suggested botulism. The detection of a neurotoxigenic organism in the feces of these patients with neurological findings consisting of bulbar palsies, flaccid paralysis, and intact sensorium further confirmed the diagnosis of botulism [3, 8].

Although no source of infections was identified, these 2 cases are believed to have resulted from intestinal infection by neurotoxigenic clostridia with in vivo production of botulinum toxin. Several lines of evidence support this idea. First, epidemiological and laboratory investigations did not identify any foods eaten by the 2 patients in the days prior to illness that contained botulinum toxin. Second, the persistence of the organism in fecal samples from the 2 patients taken on day 11 and 16, respectively, after the disappearance of constipation and the resumption of evacuation, and in 1 case (patient 2) even after the cessation of diarrhea, is evidence for colonization of the intestinal tract.

Third, type E toxin-producing organisms in the feces of the 2 patients were detected relatively long after onset, compared with the total duration of the illness, which in both cases was ~3 weeks. Other authors [22, 23] report persistent isolation of C. botulinum type A and B in both neonatal and adult patients’ feces for as long as 30 days after hospital admission. However, when the etiologic agent was C. baratti, fecal excretion lasted only 14 days [3].

In addition, the absence of the toxin in fecal samples obtained on days 16 (patient 1) and 9–11 (patient 2) indirectly confirms our conclusion that these were not classic cases of foodborne botulism, in which the fecal persistence of toxin for a number of days has been documented [24, 25]. Before hospitalization, the patients had not undergone any surgical or pharmacological treatment that might have modified their intestinal anatomy or flora. However, a congenital abnormality of the small intestine, Meckel’s diverticulum, which is rare in the general population (1%–4%), was found in both patients at surgery for suspected

### Table 3. Characteristics of neurotoxigenic organisms isolated from patients (ISS CL 86 and CL 109), and type strains of Clostridium botulinum and Clostridium butyricum type E.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CL 86</th>
<th>CL 109</th>
<th>C. butyricum</th>
<th>Type E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction on egg yolk agar</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lipase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lecitinase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Fermentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Ribose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Trehalose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Esculin hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatinase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Bot. toxin genea</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Bot. toxin productiona</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Metabolic volatile acids</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acetic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Butyric</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**NOTE.** Bot, botulinum; ISS, Istituto Superiore di Sanità; W, weak reaction; –, negative; +, positive.

*a* Type E.

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### Table 4. Susceptibility to antibiotics of 4 neurotoxigenic strains (ISS CL 86, CL 109, CL 20, and CL 21) and 2 nonneurotoxigenic strains (CL 19 and ATCC 19398) of Clostridium butyricum.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>CL 86</th>
<th>CL 109</th>
<th>CL 20</th>
<th>CL 21</th>
<th>CL 19</th>
<th>ATCC 19398</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol, 30 µg</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tetracycline, 30 IU</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Metronidazole, 5 µg</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Erythromycin, 15 IU</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Penicillin, 6 µg</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Clindamycin, 2 µg</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**NOTE.** ATCC, American Type Culture Collection; ISS, Istituto Superiore di Sanità; –, negative; +, positive.
appendicitis. Meckel’s diverticulum frequently contains hetero-
topic tissue, the presence of which may cause complications and increased morbidity [26]. This anatomic anomaly was also
found in 1 patient with infant botulism caused by the same
organism, neurotoxigenic type E *C. butyricum* [2].

What distinguishes the 2 cases described here from other
cases of adult infectious botulism is not so much the neuro-
logical profile but the abdominal and microbiological aspects,
particularly the “acute appendicitis” presentation, the implica-
tion of type E toxin, and the isolation of neurotoxigenic *C.
butyricum*.

The 2 cases reported herein are the first cases of adult in-
fected intestinal botulism in which neurotoxigenic *C. butyricum* has
been implicated. Previously, we reported 2 cases of infant botulism caused by this organism [2]. Thirteen cases of infectious
intestinal botulism have occurred in Italy since 1984. Seven were
caused by *C. botulinum* type B, 2 by *C. botulinum* type A, and
4 by *C. butyricum* type E. Recently, neurotoxigenic *C. buty-
ricum* type E was associated with an outbreak of foodborne
botulism in China [5].

The 4 Italian strains of neurotoxigenic *C. butyricum* from 2
previous and 2 recent cases matched exactly in phenotypic fea-
tures, susceptibility to antibiotics, and genotypic characteristics,
but differed from Chinese strains in their ability to ferment
arabinose. The isolation of neurotoxigenic *C. butyricum* in Italy
10 years after its initial appearance here suggests a wider dis-
tribution in our country than initially thought. Although the
previous 2 strains were found in the same city (Rome), the 2
strains that are the subject of this report were isolated a year
apart in adjacent regions of northern Italy (Emilia-Romagna
and Veneto).

Our surveys of environmental samples, commercially canned
vegetables (olives, mushrooms), and dairy products related to
foodborne botulism cases have not found neurotoxigenic *C.
butyricum* type E to be present. The sources of the organism
responsible for the Italian adult and infant cases of *C. butyricum*
intestinal botulism remain unknown. Recently, environmental
investigation of a vast area of lakeshore in China that sought
the source of neurotoxigenic *C. butyricum* involved in a food-
borne outbreak identified this organism in soil specimens from
3 of 6 sampling regions [27].

*C. butyricum* is mainly a ground bacterium that has been
isolated from soil, the rumen of calves, and animal and human
feces [16]. *C. butyricum* is frequently found in the intestine of
neonates under both physiological and pathological conditions
[28–31]. The human intestinal habitat does not seem to present
barriers to its implantation, in contrast to *C. botulinum*, for
which the normal endogenous microflora normally constitutes
a barrier to adult intestinal colonization [32]. However, when
the pH of the medium drops to <5, [33] growth of *C. butyricum*
stops. This growth-limiting factor should also be effective
against toxigenic *C. butyricum*, which differs from the nontox-
igenic *C. butyricum* only in presence of the neurotoxin gene in
its genome [34].

![Figure 1. Pulsed-field gel electrophoresis (PFGE) analysis of *C. butyricum* strains after digestion of genomic DNA with *Mlu*I (A) and *Ksp*I (B). Lane 1, molecular standard (PFGE marker I, λ-Leiter; Boehringer Mannheim); lane 2, strain ISS [Istituto Superiore di Sanità] CL 20; lane 3, CL 21; lane 4, CL 86; lane 5, CL 109; lane 6, ATCC [American Type Culture Collection] 19398 (*C. butyricum* nontoxigenic).](image-url)

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It is likely that the involvement of these unusual neurotoxigenic *C. butyricum* strains in intestinal colonization might have been responsible for the associated gastrointestinal pathology (gas and liquid in the intestinal loops, hemorrhagic enteritis) in these patients. *C. butyricum* can use lactose and produce large amounts of gas and butyrate, which promote the pathological manifestations [11].

*C. butyricum* has been incriminated in several cases of necrotizing enterocolitis [35–37], and the role played by this microorganism has been investigated. Several strains isolated from patients have been tested on various cell lines, and necrotizing enterocolitis has been produced in experimental monoxenic birds [38].

The 2 cases reported here indicate that intestinal colonization with toxigenic *C. butyricum*, as with *C. botulinum* and *C. baratii*, is not limited to infants but may also occur in adults. However, the predisposing features do not seem to be the same: the presence of Meckel’s diverticulum in 3 of the 4 cases in which neurotoxigenic *C. butyricum* has been isolated leads us to believe that this anatomic anomaly may constitute a risk factor. Meckel’s diverticulum may serve to localize the neurotoxigenic clostridia and assist in their multiplication. The reexamination of medical records from other cases of intestinal toxemia botulism, both adult and infant, might help clarify a possible predisposing role played by Meckel’s diverticulum. Finally, neurotoxigenic *C. butyricum* strains should be sought in environmental and food surveys of the distribution of botulinum spores in countries other than Italy and China.

Acknowledgment

We wish to dedicate this work to the memory of Dr. Charles Hatheway, whose scientific and human support has been precious and unforgettable.

References


Addendum. A new case of type E infant botulism came to our attention. This case was in a 5-month-old girl living in Mestre (province of Veneto). We isolated from the patient’s feces a neurotoxigenic organism, classified as C. butyricum, by sugar fermentation pattern and gas-liquid chromatographic analysis of metabolic products, that produces a type E botulinum-like neurotoxin.