Fatal Necrotizing Colitis Following a Foodborne Outbreak of Enterotoxigenic *Clostridium perfringens* Type A Infection

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**Background.** Enterotoxigenic *Clostridium perfringens* type A is the third leading cause of foodborne disease in the United States, resulting annually in an estimated 250,000 cases of a typically mild, self-limiting gastrointestinal illness.

**Methods.** A retrospective cohort study was conducted to determine the cause of a small cluster of cases of gastrointestinal illness, which included cases of severe necrotizing colitis. Participants in the study consisted of residents and staff of a residential care facility for the mentally ill in Oklahoma (n = 20). An inspection of food preparation and food storage areas of the residential care facility was conducted as part of an environmental investigation. The investigation included extensive microbiological and molecular testing of the *C. perfringens* isolates and tissue specimens collected at autopsy.

**Results.** A total of 7 (3 confirmed and 4 probable) cases of foodborne enterotoxigenic *C. perfringens* type A were identified (attack rate, 3%) after the consumption of high-risk foods. Three residents developed acute necrotizing colitis; 2 of them died. Each patient with confirmed infection presented with evidence of constipation or fecal impaction. *C. perfringens* enterotoxin (CPE)-positive *C. perfringens* type A was cultured on samples from each patient with necrotizing colitis. Although statistical analyses failed to implicate a food source, the isolates carried a chromosomal *cpe* gene, which supports a foodborne origin.

**Conclusions.** This study confirms that foodborne CPE-positive *C. perfringens* type A can affect the colon, resulting in potentially fatal necrotizing colitis. Drug-induced constipation and fecal impaction, resulting in prolonged exposure of the colonic mucosal tissue to *C. perfringens* type A toxins, contributed to the development of necrotizing colitis.

Foodborne transmission of disease in the United States has been estimated to cause 13.6 million cases of illness and up to 2700 deaths annually [1]. *Clostridium perfringens* isolates are known to cause 2 very different clinical syndromes: the relatively mild gastroenteritis due to infection with type A isolates and the very serious but rare human necrotic enteritis due to infection with type C isolates [2]. Enterotoxigenic *C. perfringens* type A is the third leading cause of foodborne disease in the United States and is estimated to cause nearly 250,000 cases of illness per year [1]. Among all foodborne pathogen infections, *C. perfringens* type A infection has one of the lowest hospitalization rates (3 hospitalizations per 1000 cases) and death rates (5 deaths per 10,000 infections) [1].

A small percentage (1%–5%) of *C. perfringens* type A isolates produces an enterotoxin (CPE) in the small intestine after consumption of a food containing at least $10^8$–$10^7$ bacteria [3–6]. The binding of the CPE in the small intestine is typically associated with mild, self-limiting gastrointestinal illness characterized by an incubation period of 8–24 h and acute abdominal pain, diarrhea, and nausea lasting 12–18 h. Fatal cases of *C. perfringens* foodborne disease are rare and are thought to be limited to elderly, very young, debilitated, or chronically ill persons [4, 6].
On 24 November 2001, a residential care facility (RCF) for the mentally ill in Oklahoma notified the Oklahoma State Department of Health that 2 residents had died and another resident was hospitalized due to a gastrointestinal illness. A large Thanksgiving meal had been consumed 2 days prior to these events. We initiated an investigation to determine the extent and cause of this disease cluster. We report 3 cases of severe necrotizing colitis resulting from a foodborne outbreak. To our knowledge, we report the first 3 cases of necrotizing colitis resulting from a foodborne outbreak of C. perfringens type A infection. In addition, the pathology of disease described in this article involves the colon, which previously has not been shown to be involved with a CPE-positive C. perfringens type A etiology.

METHODS

We conducted a retrospective cohort study among residents and staff of the RCF and gathered exposure information, medical histories (including prescription information), acute disease symptomatology, and laboratory information on a standardized data collection instrument. We gathered clinical and laboratory data on hospitalized and deceased individuals from hospital medical records. χ² Tests, relative risks, and 95% CIs were calculated with EpiInfo software, version 6.04d (Centers for Disease Control and Prevention), in accordance with the methods of Mehta et al. [7]. Stool specimens were obtained when available. A standard autopsy and toxicology analysis was performed for each fatal case by the Office of the Chief Medical Examiner, and tissue specimens were obtained.

A licensed environmental specialist conducted an environmental investigation, which included inspection of the RCF’s food preparation and food storage areas. The RCF food handler was interviewed to obtain a thorough food preparation history focused on the food items prepared for the large Thanksgiving meal.

A confirmed case was defined as a positive result of laboratory culture for CPE-positive C. perfringens type A in samples from a person who was a resident of the RCF who had an onset of symptoms from 22 November to 24 November 2001; a probable case was defined as vomiting or diarrhea (≥3 loose stools in a 24-h period) experienced by a resident of the RCF who had a reported onset of symptoms from 22 November to 24 November 2001.

Fecal specimens from patients were submitted for anaerobic culture and identification with the use of the RapID ANA II System (Innovative Diagnostic Systems) in accordance with the instructions of the manufacturer. Isolates confirmed as C. perfringens were tested for CPE with the use of a reverse passive latex agglutination (PET-RPLA) CPE toxin detection kit (Oxoid) in accordance with the instructions of the manufacturer.

The ability of the isolates to express the cpe gene was then further confirmed using Western blot analysis, as described elsewhere [8]. C. perfringens toxin type was assigned using a multiplex PCR assay [9]. A duplex PCR assay was used to determine whether the cpe genes in these isolates were either chromosomal or plasmid-borne [10]. The clonality of isolates from cases was examined by an unpublished PCR procedure that tests for the presence and length of 4 short repetitive sequences, or variable number of tandem repeats (VNTRs).

Sections of affected colon collected at autopsy were cut and were hematoxylin and eosin–stained and Gram-stained for histological analysis. The tissue sections were then tested for the presence of CPE by immunohistochemical staining. From the colons of 2 patients, 4-µm-thick paraffin sections were processed with an indirect immunoperoxidase technique for CPE, by use of the Dako EnVision kit (Dako), in accordance with the manufacturer’s instructions, and by use of a monoclonal antibody against CPE. The colon of a rabbit inoculated experimentally with CPE was used as a positive control. The colon of a rabbit inoculated with phosphate-buffered saline (pH, 7.2) and the colon from a human were used as negative controls. The following case reports include information regarding each of the confirmed, outbreak-associated cases of CPE-positive C. perfringens type A infection.

CASE REPORTS

Patient 1. A 39-year-old white woman with a history of paranoid schizophrenia, depression, hypothyroidism, hypertension, and chronic constipation experienced an acute onset of diarrhea, nausea, and vomiting on 22 November 2001. The next morning, she continued to experience diarrhea and was found incontinent, with large amounts of liquid stool. Her condition rapidly deteriorated, and, ∼17 h after the onset of symptoms, the patient became unresponsive, went into cardiopulmonary arrest, and was pronounced dead on arrival at the hospital.

At autopsy, the small and large bowels were notably distended by copious gray-tan to dark-brown fluid and some semisolid fecal material. There was no perforation or peritonitis. Within the large bowel, the mucosa of an ∼30.5-cm–long segment of the transverse colon was necrotic. This segment was distinctly black in color, in contrast to the remainder of the colonic mucosa, which was grossly unaffected. On histological examination, the colon showed severe multifocal to coalescent acute mucosal necrosis that involved the superficial epithelium and lamina propria, with complete loss of the colonic epithelium. There was also severe diffuse hemorrhage and inflammatory infiltrate consisting of mainly neutrophils and a few lymphocytes, plasma cells, and macrophages. The inflammation involved all layers of the colon, including the serosa. A large number of gram-positive rods with rounded ends were present...
on the surface of the mucosa and were mixed with sloughed epithelial cells and inflammatory exudates in the intestinal lumen and deep in the mucosa. Unaffected areas of the small and large bowels were essentially unremarkable on microscopic evaluation. Immunohistochemical staining of colonic tissue specific to CPE was not observed, perhaps because of the complete loss of the colonic epithelium.

The patient had been a resident of the RCF for >2 years. Her daily multidrug treatment regimen consisted of topiramate, ranitidine, benztropine mesylate, clozapine, sertraline, oxcarbazepine, furosemide, hydroxyzine hydrochloride, diphenhydramine hydrochloride, and trazodone.

**Patient 2.** A 58-year-old black man with a history of schizophrenia, hypertension, emphysema, and chronic constipation had an acute onset of abdominal pain, diarrhea, and vomiting midmorning on 23 November 2001. His condition was treated at a local hospital, and he was released with an order to increase oral fluid intake. Two hours later, he was disoriented and complained of symptoms consistent with acute gastrointestinal illness. He was found to be incontinent, with copious amounts of liquid stool and a large mass of hard feces, and he returned to the hospital. His vital signs were normal, and his abdomen was soft and without tenderness and had no evidence of obstruction. The results of standard laboratory tests, including a complete blood cell count with differential and basic metabolic profile, were unremarkable. His condition was treated intravenously with 1 L of normal saline and 12.5 mg of promethazine hydrochloride, and he was released. The patient continued to experience diarrhea and complained of left-side abdominal pain. At 7 h after his return from the hospital and ~24 h after the onset of illness, the patient was found to have no pulse and was pronounced dead.

At autopsy, necrosis was observed on the colonic mucosa, beginning with the splenic flexure and including the descending colon and portions of sigmoid, with relative discrete delineation from unaffected areas (figure 1). The left half of the transverse colon and the descending colon contained a moderate amount of watery, turbid, yellow-brown fluid. The small bowel and segments of the large bowel consisting of the ascending colon and the right half of the transverse colon were unremarkable. On histological analysis, the finding of severe multifocal to coalescent acute mucosal necrosis was consistent with the findings for case 1. However, a few areas of remaining colonic epithelium were observed. The inflammation involved all layers of the colon, including the serosa. A large number of gram-positive rods with rounded ends were present on the surface of the mucosa and were mixed with sloughed epithelial cells and inflammatory exudate in the intestinal lumen and deep in the mucosa. Immunostaining specific to CPE was observed as clumps on the surface of the mucosa and included most of the rods seen in the mucosa.

The patient had been a resident of the RCF for 5 years. His daily multidrug treatment regimen consisted of trazodone, risperidone, olanzapine, carbamazepine, citalopram hydrobromide, benztropine mesylate, lorazepam, oxybutynin chloride, desmopressin acetate, hydrochlorothiazide, and ibuprophen.

**Patient 3.** A 59-year-old black man with a history of schizophrenia and chronic constipation complained of gastrointestinal illness consisting of nausea, vomiting, and diarrhea midmorning on 23 November 2001. He defecated a large mass of hard feces and copious amounts of loose stool. He was transported to the hospital, where a complete blood cell count with differential and basic metabolic profiles revealed a WBC count of $12.6 \times 10^3$ cells/μL, with 83% neutrophils and 4.6%
Table 1. Frequency of exposure to foods among residents and staff of the Oklahoma residential care facility for the mentally ill in November 2001.

<table>
<thead>
<tr>
<th>Food</th>
<th>No. of cases (%)</th>
<th>No. of noncases (%)</th>
<th>RR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey</td>
<td>7 (100)</td>
<td>12 (92)</td>
<td>Undefined^a</td>
<td>NS</td>
</tr>
<tr>
<td>Ham</td>
<td>7 (100)</td>
<td>12 (92)</td>
<td>Undefined^a</td>
<td>NS</td>
</tr>
<tr>
<td>Dressing</td>
<td>6 (86)</td>
<td>11 (85)</td>
<td>1.06 (0.19–5.94)</td>
<td>NS</td>
</tr>
<tr>
<td>Turkey gravy</td>
<td>5 (71)</td>
<td>10 (77)</td>
<td>0.83 (0.23–3.03)</td>
<td>NS</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>5 (71)</td>
<td>12 (92)</td>
<td>0.44 (0.15–1.31)</td>
<td>NS</td>
</tr>
<tr>
<td>Turkey sandwich</td>
<td>4 (57)</td>
<td>5 (42)b</td>
<td>1.48 (0.45–4.90)</td>
<td>NS</td>
</tr>
<tr>
<td>Ham sandwich</td>
<td>3 (43)</td>
<td>5 (42)b</td>
<td>1.03 (0.31–3.38)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of individuals who consumed the food item. Results are given for a total of 7 cases and 13 noncases. NS, not significant. RR, relative risk.

^a Both the RR and 95% CIs are undefined.
^b Data were available for only 12 persons.

The patient was repeatedly hospitalized due to complaints of gastrointestinal illness and then released. On physical examination, his abdomen was soft, with slight tenderness and distention. Subsequent complete blood cell count and metabolic profiles remained within normal limits. A flexible sigmoidoscopy performed on 28 November 2001 indicated severe pseudomembranous colitis and possible ischemic bowel.

The patient was a resident of the RCF for 5 years. His daily multidrug treatment regimen consisted of fluoxetine hydrochloride, benztropine mesylate, vitamin E, olanzapine, and lorazepam.

RESULTS

All residents and staff of the RCF consumed a large Thanksgiving meal at ~12:00 P.M. on 22 November 2001. The food items served included turkey, ham, sweet potatoes, dressing, and turkey gravy. Improper food-handling practices were identified in the storage and preparation of the large number of turkeys served to the residents and staff of the RCF.

Of the 26 residents and staff of the RCF, 20 provided exposure and acute illness histories. Seven cases (3 confirmed and 4 probable) of foodborne CPE-positive C. perfringens type A infection were identified (attack rate, 35%); no staff members developed the illness. The median age at onset was 48 years (range, 30–59 years); 4 (57%) of the 7 infected patients were female. The onset of illness among patients ranged from 22 November through 23 November 2001. Symptoms associated with illness included diarrhea (100% of infection persons), abdominal cramps (100%), vomiting (71%), and fever (14%). The median incubation period for confirmed cases was 18 h (range, 10–22 h) after the consumption of the Thanksgiving meal. Statistical analysis failed to implicate a food source (table 1), and no food items from the Thanksgiving meal were available for bacterial culture. Three (43%) of the patients developed severe bowel necrosis, which resulted in 2 deaths.

C. perfringens was cultured from each patient presenting with necrotizing colitis. A Western blot analysis established that isolates from each confirmed case were capable of producing CPE, validating the initial positive identification of CPE expression by PET-RPLA. Each isolate had a positive result of PCR for the CPE gene and the alpha toxin (p/c) gene but not for the β, ε, or τ toxins genes, which confirmed that the bacteria was CPE-positive C. perfringens type A (figure 2). In addition, we determined that these isolates carried a chromosomal cpe gene (figure 3). PCR analyses of VNTRs suggested that isolates from each confirmed case were closely related, if not identical. No fecal specimens from probable cases were provided for laboratory testing.

DISCUSSION

The 3 cases of necrotizing colitis resulting from a foodborne outbreak of CPE-positive C. perfringens type A are remarkable, because CPE has not previously been shown to affect the human
The 3 cases presented here differ from the fatal cases attributed to enterotoxigenic (or CPE-positive) *Clostridium perfringens* type A in the literature, which are typically due to dehydration in elderly, very young, debilitated, or chronically ill persons [4, 6]. Despite their mental illness, the patients reported in the current study were not otherwise physically debilitated or immunocompromised.

We believe that constipation and fecal impaction may have been risk factors for developing necrotizing colitis after the ingestion of contaminated food. Constipation likely resulted from the anticholinergic effects of psychiatric medications. The distinct delineation of the necrotic and unaffected mucosal tissues seen on examination of the colon is thought to be the result of a fecal impaction preventing the expulsion of CPE through the usual protective effects of diarrhea; thus, the prolonged exposure of the tissue to the CPE (and perhaps to other toxins of *C. perfringens* type A) resulted in the severe necrotic process. The cause of death in each of the fatal cases was determined to be severe colon necrosis. Although disease due to the release of CPE after foodborne transmission of CPE-positive *C. perfringens* is typically mild, animal experiments provide support for the potency of CPE in a closed gastrointestinal tract [11].

*C. perfringens* type A is a ubiquitous organism that is part of the normal fecal flora of humans [3, 4]; therefore, a positive culture result does not confirm a *C. perfringens* type A etiology of disease. Considerable epidemiologic evidence implicates CPE as the virulence factor responsible for most (if not all) of the diarrheal and cramping symptoms associated with *C. perfringens* type A foodborne disease [12]. CPE detection in samples from the 3 patients provides definitive evidence for the confirmation of a CPE-positive *C. perfringens* type A etiology [13–15]. Additionally, immunostaining specific to CPE at the site of necrosis was observed for the patient with remaining enterocytes, which strongly suggests that this toxin was involved in the pathogenesis of the condition.

CPE-positive *C. perfringens* type A isolates are classically associated with foodborne disease [1, 4–6, 16]. However, during the past 15 years, CPE-positive *C. perfringens* type A isolates have become linked to several cases of nonfoodborne gastrointestinal disease, [17–21] including up to 5%–20% of all antibiotic-associated diarrhea [22]. It is now evident that the *cpe* gene typically has a chromosomal location in CPE-positive *C. perfringens* type A transmitted through food [21, 23, 24] but is plasmid-borne in CPE-positive *C. perfringens* type A not transmitted through food [17, 21]. Disease from nonfoodborne *C. perfringens* type A occurs almost exclusively in elderly persons and is typically more severe, with a longer duration of illness [19, 25].

We believe that this outbreak of CPE-positive *C. perfringens* type A–associated disease was foodborne. The acute onset of illness among residents of the same RCF suggests a point-source foodborne disease outbreak and was consistent with the observed incubation period after the consumption of turkey, a common food vehicle for CPE-positive *C. perfringens* [15, 26, 27]. In addition, the isolates were found to carry the *cpe* gene on the chromosome and not on the plasmid, which provides additional support that the source of the disease was a food product [10]. *C. perfringens* foodborne isolates with the *cpe* gene carried on the chromosome are more heat resistant than are those with the gene carried on the plasmid; thus, their survival is favored in inadequately cooked foods.

Conducting an epidemiologic investigation among a cohort of persons who are independent in feeding and toileting yet who have difficulty communicating exposure and illness history presented several limitations. Six residents unable to answer questions about symptoms and food history were excluded from the analysis, which limited additional case findings and reduced the sample size for analysis of exposure variables. In addition, there were no leftover food items available for testing. These limitations prevented us from identifying a specific food source.

These case reports demonstrate the potential seriousness of *C. perfringens* foodborne disease, particularly in a medicated population at risk of chronic constipation. The bowel health of patients receiving medications that may cause constipation should be closely monitored, and stool softeners should be prescribed as needed. It is likely that most outbreaks of *C. perfringens* foodborne disease occur in institutions and food service facilities that cook large amounts of food well in advance of serving [4]. Outbreaks of foodborne disease, including those resulting from CPE-positive *C. perfringens* type A, are preventable through the use of safe food-handling practices. Food
handlers in these facilities should be trained in food safety. In addition, patients presenting with necrotizing colitis of unknown cause should be evaluated for a CPE-positive C. perfringens type A etiology of disease.

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