Fatal Pseudomembranous Colitis Associated with a Variant *Clostridium difficile* Strain Not Detected by Toxin A Immunoassay

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Background: **Many clinical laboratories use toxin A immunoassays to test for *Clostridium difficile*.**

Objective: **To describe the clinical course of a patient infected with a toxin variant strain of *C. difficile* that was not detected by toxin A immunoassay; to genetically characterize this strain; and to estimate the number of laboratories that use only toxin A immunoassays.**

Design: **Case report, molecular investigation, and laboratory survey.**

Setting: **Tertiary care hospital in Chicago, Illinois.**

Patient: **An 86-year-old man.**

Measurements: **Restriction endonuclease analysis, polymerase chain reaction, and survey of regional clinical laboratories.**

Results: **An elderly hospitalized man died of advanced pseudomembranous colitis. Four stool specimens submitted over a 2-month period had tested negative on toxin A immunoassay, but a strain of *C. difficile* with a 1.8-kb deletion of the toxin A gene was recovered from each specimen. This strain, identified as restriction endonuclease analysis type CF4, is closely related to a widely disseminated variant, toxinotype VIII. Toxin A immunoassay was the only test being performed for detection of *C. difficile* at 31 of 67 (46%) regional clinical laboratories.**

Conclusions: **Toxin A variant strains of *C. difficile* cause serious disease and are undetectable in clinical laboratories that use only toxin A immunoassays for *C. difficile* testing.**

Ann Intern Med. 2001;135:434-438. **www.annals.org**

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Clostridium difficile infection should be a primary diagnostic consideration in any patient with diarrhea who has been hospitalized and has recently received antibiotics. Detection of toxin in patient stool specimens is the most important laboratory evidence for confirming a diagnosis of *C. difficile* diarrhea, although reliance on any one test may be insufficient to exclude the diagnosis (1). *Clostridium difficile* produces two large single-unit toxins (toxins A and B), which share significant functional domain homology and act by glycosylation of small guanosine triphosphate–binding proteins that are involved in cell cytoskeleton organization (2). Commercial immunoassays have been developed that detect toxin A by using the monoclonal antibody PCG-4, which recognizes epitopes encoded by a region of highly repetitive DNA sequences in the 3′ end of the toxin A gene (3). In general, these immunoassays have a similar specificity but a somewhat decreased sensitivity compared with cell-culture cytotoxin assays, which primarily detect the effects of toxin B (1).

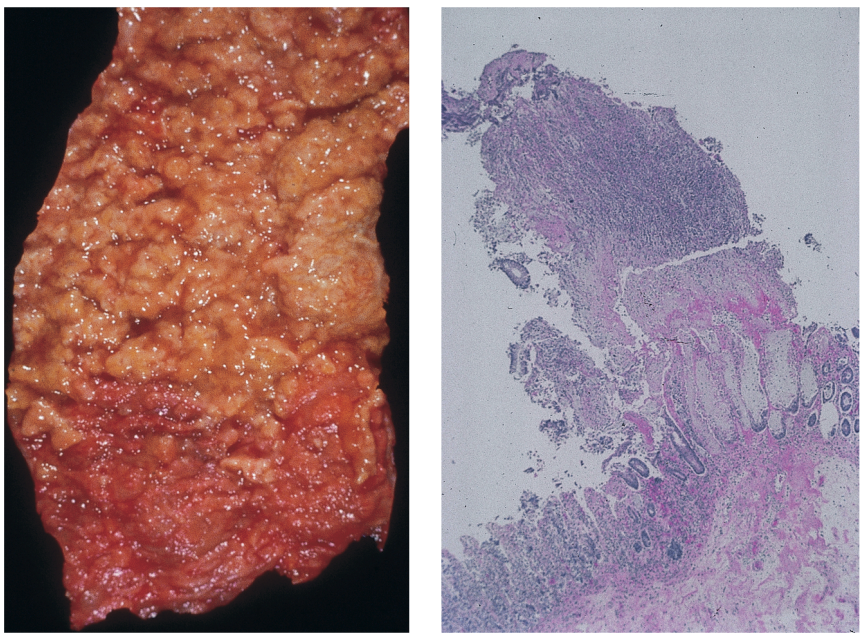
In addition to using toxin testing, the clinical laboratory at our hospital in Chicago, Illinois, has increased the sensitivity of diagnostic testing for *C. difficile* disease by adding a culture for *C. difficile*. Toxin assays are also performed in vitro on the culture supernatants of recovered isolates to screen for nontoxigenic (nonpathogenic) strains. In 1998, the cell-culture cytotoxin assay that had been used at our laboratory was replaced with an immunoassay for toxin A (Clearview C. DIFF A, Wampole Laboratories, Division of Carter-Wallace, Inc., Cranbury, New Jersey) after a prospective study showed that this toxin A immunoassay had a sensitivity and specificity similar to those of the cytotoxin assay (4).

We report on a patient who died of pseudomembranous colitis that was caused by a strain of *C. difficile* undetected by repeated toxin A immunoassays. We genetically characterize this strain of *C. difficile* and estimate the number of clinical laboratories in our region with test strategies that will not detect this variant strain.

Case Report

An 86-year-old man with multiple cardiac and pulmonary problems was admitted to the hospital for treatment of presumed inflammatory bowel disease. During a previous hospitalization 2 months earlier, the patient had developed diarrhea and had been treated for pneumonia with antibiotics. At that time, findings on stool specimen testing were negative for toxin A but the culture was positive for *C. difficile*. The recovered *C. diffi-*

Figure. **Gross findings at autopsy performed 2 months after symptom onset.**



Left. Diffuse pseudomembranous colitis is demonstrated with characteristic yellow patches becoming confluent in some areas. The pseudomembranes did not involve the small intestine. **Right.** Microscopic examination of pseudomembranous plaques at autopsy (hematoxylin–eosin stain; magnification, ×100). Sharply defined eruptions of exudate (composed of neutrophils, mucus, fibrin, and cellular debris) adhere to the damaged mucosal surface.

cile isolate tested negative for toxin A production in vitro (using the same toxin A immunoassay used on the stool specimen) and was reported as nontoxigenic. The patient was subsequently evaluated on several occasions for lower abdominal cramps and recurrent diarrhea. Findings on colonoscopy and abdominal computed tomography were suspicious for *C. difficile*–associated pseudomembranous colitis, but this diagnosis was dismissed because of two additional stool specimens that were negative for toxin A and were culture positive for a presumed nontoxigenic *C. difficile* strain.

Two months after the initial onset of symptoms, the patient returned to the hospital with diarrhea, fever (37.4 °C [99.3 °F]), abdominal tenderness, and an elevated leukocyte count (14.2×10^9 cells/L). The patient was treated with steroids for presumed inflammatory bowel disease, partly because a recent extensive gastrointestinal evaluation (which had included a stool culture for routine enteric pathogens and an ova and parasite examination) was unrevealing. The patient's diarrhea improved, but the lower abdominal cramping persisted. On the 3rd day of hospitalization, the patient experienced cardiopulmonary arrest, and attempts to resuscitate him were unsuccessful.

Autopsy showed diffuse pseudomembranous colitis (**Figure,** *left panel*). A stool specimen obtained at the time of autopsy was negative for toxin A but was culture positive for a presumed nontoxigenic strain of *C. difficile*. After the autopsy report was received, the four clinical isolates were tested in vitro by using a cell-culture cytotoxin assay. All isolates tested positive for cytotoxin. No specific treatment for *C. difficile* diarrhea had been administered at any time during the patient's illness.

Molecular Investigation

All four clinical isolates were identical by using *Hin*dIII-restriction endonuclease analysis typing (5) (data not shown) and were designated as restriction endonuclease analysis type CF4. The restriction pattern of the four isolates matched that of a group of highly related isolates in our clinical isolate collection (5); this group of related isolates, designated as CF, is known to be toxin variant. Although strains in the CF group pro-

Table. **Diagnostic and Clinical Characteristics of *Clostridium difficile* Strains with Different Toxigenic Potential**

C. difficile Strains	Diagnostic Methods			Potential for *C. difficile* Disease?	Prevalence, %*
	Cytotoxin Assay	Toxin A Immunoassay	Culture		
Toxigenic (toxin A–positive, toxin B–positive) strains	Positive	Positive	Positive	Yes	90
Toxin A–negative, toxin B–positive strains†	Positive‡	Negative	Positive	Yes	Unknown
Nontoxigenic strains	Negative	Negative	Positive	No	10

* Approximate prevalence in hospitalized patients with antibiotic-associated diarrhea and stool cultures positive for *C. difficile* (7).
† Variant *C. difficile* strains that do not produce toxin A, such as the restriction endonuclease type CF4 strain recovered from the patient described in this report.
‡ Cytopathic effect differs slightly but neutralizes with standard *C. difficile* antitoxin.

duce toxin B and are cytotoxic, these variant strains do not produce toxin A and are characterized by a 1.8-kb deletion in the 3′ end of the toxin A gene (6) (**Table**). Thus, we amplified the 3′ end of the toxin A gene by performing polymerase chain reaction. The amplification was done on DNA that had been isolated and purified from the *C. difficile* isolates of patients and controls, as previously described (6), by using the primers A-u2 and A-d1-b (the primers start at positions 5301 and 8136, respectively, on the toxin A gene). The amplified product from the four isolates recovered from our patient was 1.8 kb smaller than the product from amplification of the standard toxigenic strain VPI 10463; the size of the amplified product was identical in size to the amplified product from the toxin B–positive, toxin A–negative variant strain, type CF2 (6) (data not shown).

To identify additional CF isolates, we screened our collection of more than 5000 clinical isolates (6). By using restriction endonuclease analysis, we identified 58 variant CF isolates (1.7% of the 3445 total isolates typed), including 3 additional CF4 isolates from patients at two Veterans Affairs hospitals (in Minneapolis, Minnesota, and in Chicago) and a county hospital (in Chicago). At that time, the clinical laboratory at each of the three hospitals had been using both cytotoxin assays and culture for *C. difficile* testing. In all three patients, results on stool cytotoxin assays were positive and *C. difficile* diarrhea was diagnosed.

Telephone Survey

In September 2000, we surveyed all hospital-based laboratories in the Chicago metropolitan area to determine the type of *C. difficile* testing being performed. Immunoassay for toxin A was the only test being used to detect *C. difficile* in 31 of the 67 (46%) laboratories surveyed. Two laboratories were using a latex agglutination test for nontoxin *C. difficile* antigen, and the remaining laboratories performed either immunoassay for both toxins A and B or a cell-culture cytotoxin assay.

Discussion

Reliance on a single laboratory test despite epidemiologic and clinical clues that were highly suggestive of *C. difficile* infection contributed to the death of this patient. The patient initially presented with abdominal pain and diarrhea in the hospital after receiving antibiotic therapy for pneumonia. Although findings on colonoscopy and abdominal computed tomography were highly suggestive of *C. difficile*–associated pseudomembranous colitis, the diagnosis was not made. Laboratory testing of stool specimens for *C. difficile* toxin is an accepted method for the diagnosis of *C. difficile* diarrhea. However, these tests are not highly sensitive compared with stool culture, and a negative result on immunoassay or on cytotoxin assay should not be used to exclude the diagnosis of *C. difficile* diarrhea (8).

Several *C. difficile* strains with variations in the pathogenicity locus of the toxin gene have been reported (9–11). Rupnik and colleagues classified these variations into 15 toxinotypes by using molecular techniques (12). Toxinotype VIII strains are characterized by a 1.8-kb deletion in the toxin A gene and altered restriction sites in the toxin B gene. The toxinotype VIII strains do not produce a functional toxin A and are undetectable by toxin A immunoassays (13). Until recently, reports had suggested that toxinotype VIII strains, which also correspond to serogroup F [14], were nonpathogenic (10, 14, 15).

We recently characterized a toxin variant of a *C. difficile* strain that we designated as restriction endonu-

clease analysis type CF2. The CF2 strain was found in specimens recovered from seven patients at a Veterans Affairs hospital in Minneapolis; five of the seven patients had documented cases of *C. difficile* diarrhea (6). Despite producing no toxin A and having genotypic characteristics nearly identical to those of strain 1470, which is the prototype strain for toxinotype VIII, type CF2 is cytotoxic and can cause disease in hamsters (although with lower colonization efficiency and mortality compared with other, fully toxigenic strains of *C. difficile* [16]). Recently, a nosocomial outbreak of *C. difficile* diarrhea due to a toxinotype-VIII variant strain was reported at a hospital in Winnipeg, Manitoba, Canada (17).

Toxin-variant strains of *C. difficile* that appear identical or similar to toxinotype VIII are more widespread than was previously recognized (6, 17–19). Moreover, these strains can cause the full spectrum of *C. difficile*–associated diseases. A recent survey conducted by the United Kingdom National External Quality Assessment Scheme reported that 108 of 243 (44%) laboratories surveyed had been performing only toxin A testing for diagnosis of *C. difficile* (United Kingdom National External Quality Assessment Scheme. *Clostridium difficile* Survey. Distribution no. 1305. 10 January 2000). The results of the U.K. survey and of our survey of hospital-based laboratories in the Chicago metropolitan area suggest that nearly half of all clinical laboratories may be unable to detect these strains. As a result of this patient's death, the clinical laboratory at our hospital has included an in vitro cytotoxin assay for evaluation of all *C. difficile* isolates recovered from stool specimens that test negative on toxin A immunoassay. More data are needed to determine the incidence of these toxin A–negative, toxin B–positive variants before recommendations on toxin A immunoassay testing for *C. difficile* are altered (20). Clinical laboratories and clinicians who rely solely on toxin A immunoassay to diagnose *C. difficile* disease should recognize the possibility of *C. difficile* diarrhea in patients with negative test results.

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Grant Support: By the Department of Veterans Affairs Research Service and a Northwestern University Medical School Intramural Research Grant from the Northwestern Memorial Hospital Intramural Research Grants Programs.

Case Summary

A fatal case of pseudomembranous colitis was associated with a toxin A–negative strain of *Clostridium difficile*.

Several toxin A immunoassays failed to detect this toxin A–negative strain of *C. difficile*.

According to a survey of Chicago-area hospitals, nearly 50% of hospital clinical laboratories were using only toxin A immunoassay to test for *C. difficile*.

These variant strains have been recovered from patients in Europe, Canada, the United States, and Japan; however, the incidence of these strains is unknown.

A negative result for *C. difficile* on toxin assay, whether immunoassay or other, should never be used clinically to exclude the diagnosis of *C. difficile* disease if epidemiologic and clinical features are consistent with the diagnosis.

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