Septic reaction case reports

From March 2003 through December 2003, before screening, 15 septic reactions involving apheresis PLTs were reported. Twelve were assessed as high probability, 2 of which were fatal. In the same period following screening, 8 septic reactions involving apheresis PLTs were investigated and 3 were assessed as high probability. The investigation of these 3 cases did not identify any irregularities in the donor physical exam, donor health history and record, phlebotomist arm preparation technique, component sampling for culturing, or incubation of the inoculated bottle.

Case 1. An elderly man received apheresis PLTs after a difficult coronary artery bypass redo. One hour later, his temperature increased from 37.6 to 39.4°C with rigors, tachycardia, shortness of breath, and hypotension. Gram stain of the PLT unit showed Gram-positive cocci in clusters. Cultures of both the unit and the patient's blood grew coagulase-negative staphylococcus, subsequently confirmed to be identical strains of *Staphylococcus lugdunensis*. The BacT/ALERT bottle had not alarmed and the bottle indicator remained negative. Gram stain and culture of the bottle were negative. The bottle's content was noted to be slightly cloudy, however, and PLTs were seen on the Gram stain, documenting that the bottle had been inoculated. The bag was examined at the hospital for any defects that may have allowed contamination; none were found. The patient died the day after his reaction when support was withdrawn. (Note: This case was previously published by the Centers for Disease Control and Prevention.[13])

Case 2. A 15-year-old patient was admitted with fever, neutropenia, and thrombocytopenia due to "lymphosarcoma thorax." Halfway through transfusion of an apheresis PLT unit, she developed a temperature elevation from 36.7 to 39.1°C with severe rigors. A Gram stain of the unit showed many Gram-positive cocci subsequently identified as *Staphylococcus epidermidis*. The patient's post-transfusion blood cultures were also positive for *S. epidermidis*. The BacT/ALERT bottle had not alarmed and the bottle indicator remained negative. Gram stain and culture of the bottle were both negative. The patient was treated and survived.

Case 3. A 49-year-old woman was admitted with acute coronary syndrome and gastrointestinal bleeding and found to have a drug-induced thrombocytopenia. While receiving an apheresis PLT, she developed a rise in temperature from 37.1 to 39.0°C, dyspnea, chills, and a mottled skin appearance. A Gram stain and culture on the residual bag contents showed Gram-positive cocci in clusters subsequently identified as coagulase-negative *Staphylococcus*. The patient was treated and survived. The other half of this split collection had been transfused uneventfully to a patient with acute myelogenous leukemia who was medicated with diphenhydramine and acetaminophen, and was receiving vancomycin, fluconazole, metronidazole, and ceftazadine.

TABLE 4. Incubation time (hr) required for initial positive result

Sample result	Number	Mean ± SD	Median	Range
Confirmed-positive	68	19.0 ± 13.8	16.2	5.9-105.6
(Confirmed-positive*)	(67)	(17.7 ± 8.9)	(16.0)	(5.9-50.4)
False-positive due to instrument error	39	22.6 ± 20.2	20.0	0.0-117.0
False-positive due to contamination	75	34.1 ± 22.6	25.6	6.2-110.0
Indeterminate	44	48.4 ± 34.1	35.3	4.1-120.0

* Excluding one *Bacillus* contaminated unit with a time to positive of 105.6 hours.

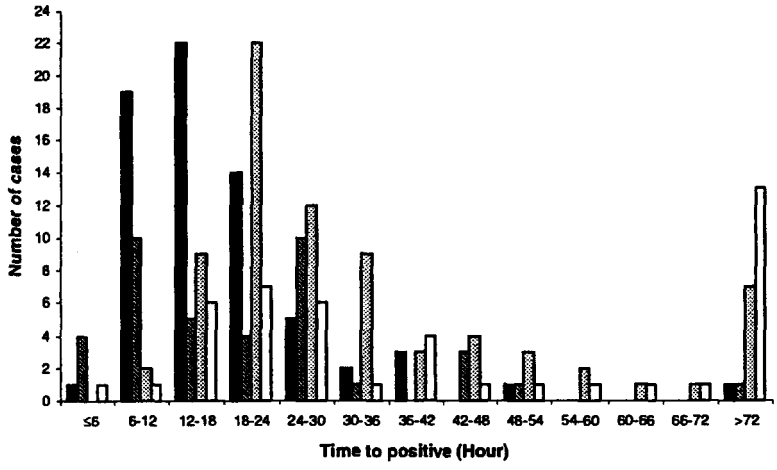

Fig. 2. Distribution of incubation time required for different types of positive reactions. (■) Confirmed-positive; (▩) false-positive (instrument); (▨) false-positive (contamination); (□) indeterminate.

DISCUSSION

Routine culturing for detection of bacterial contamination of apheresis PLTs was successfully implemented in every regional blood center of the American Red Cross. The positive rate was 1 in 1552 collections, of which 30.1 percent could be confirmed, based on reculturing and bacterial isolation, for a contaminated collection detection rate of 1 in 5157. This is significantly lower ($p = 0.0127$) than our previous report[14] of a contamination rate of 1 in 1249 PLTs collected at four regional blood centers also represented in this study. The earlier study methods were significantly

TABLE 5. Incubation time (hr) required for confirmed-positive reaction according to type of bacteria

Type of bacterium	Number	Mean ± SD	Median	Range
S. epidermidis	10	19.0 ± 2.4	19.2	16.4-22.9
Staphylococcus aureus	2	10.8 ± 1.6	10.8	9.7-11.9
Other coagulase-negative *Staphylococcus* spp.	19	23.9 ± 7.6	22.0	15.3-41.6
S. bovis	5	12.0 ± 1.5	12.2	9.8-13.7
Streptococcus viridans	6	16.5 ± 10.7	12.3	10.2-38.0
S. marcescens	4	9.9 ± 1.8	9.2	8.7-12.5
Klebsiella spp.	3	7.8 ± 1.8	7.7	6.1-9.7
E. coli	3	11.3 ± 5.8	10.6	5.9-17.5
All Gram-positive organisms*	55	19.4 ± 8.8†	16.5	8.5-50.4
All Gram-negative organisms	12	9.9 ± 3.3†	9.2	5.9-17.5

* Excluding one *Bacillus* contaminated unit with a time to positive of 105.6 hours.
† $t = 3.639$; $p = 0.0005$.

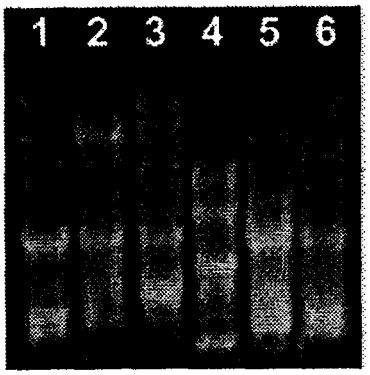

Fig. 3. Random amplified polymorphic DNA analysis on different *S. bovis* strains. (Lanes 1-3, three different strains obtained from the ATCC; Lanes 4-6, isolations from three apheresis PLT products).

different from the program designed here: whole blood–derived PLTs were tested, samples were collected 1 to 7 days after expiration, and cultures in both sheep blood agar plate and thioglycollate broth medium were performed. In the earlier study, one of the four positive cases was *Propionibacterium acnes*, which is an anaerobic organism that usually does not grow in the aerobic culture bottle of the BacT/ALERT system.[15] If this case is excluded from analysis, the difference between the pilot study and the 10-month experience is not significant ($p = 0.1295$).

In our experience, the proportion of Gram-negative bacteria (17.6%) in the confirmed-positive group is similar to what was reported by Goldman and Blajchman[15] where they compiled a list of contaminating organisms involved in PLT contamination cases from eight different reports. Although representing approximately one-fifth of contaminations, the BaCon study[17] reported that Gram-negative organisms accounted for 41 percent of deaths due to septic transfusion reactions. This is presumed to be because Gram-negative bacteria multiply rapidly, as reflected in the shorter incubation time to detection in our experience, and produce endotoxin, both of which mediate more severe clinical consequences.

Likewise, our experience and the Goldman and Blajchman[16] review show that close to 50 percent of PLT contaminations were of *Staphylococcus* spp. In our experience, *Streptococcus* spp. (26.9%) were the next most frequently implicated organisms whereas other studies have reported *Corynebacterium* species (diphtheroids). This may reflect the fact that we did not consider an organism to be confirmed as having caused contamination of the component unless it could be isolated from a second independent sample from either the original collection or at least one of the split products. Without this requirement, it would be possible for other programs to misinterpret a single positive culture for *Corynebacterium* spp., which are common sampling and handling contaminants. In our study, *Corynebacterium* spp. were isolated from none of the confirmed-positive culture bottles but were from 6.7 percent of false-positive samples (Table 3).

For our routine bacterial QC testing, only the aerobic culture bottles are used for three major reasons: 1) PLT products are stored under an aerobic condition and hence provide a poor environment for growth of strict anaerobic organisms; 2) many of the clinically significant facultative anaerobic bacteria grow under aerobic conditions; and 3) most anaerobic bacteria that would be encountered during PLT manufacture, for example, *Propionibacterium* spp., are rarely clinically significant. We also release the PLT components to transfusion as long as the culture has been negative for 12 hours, although the culture bottles are kept in the incubator until the end of product shelf life. This improves the availability of PLT products for patient use. Our internal data show that 42 percent of products are actually distributed 48 hours or more after collection. During this 10-month review, the earliest time a PLT unit was transfused before an initial positive signal by the BacT/ALERT system was 23 hours. In this case, the initial result was falsely positive owing to sampling; a repeat culture from the other half of the collection was negative. No PLT associated with a confirmed-positive screen was transfused during the period of the study. A total of 52.9 percent of confirmed-positive cases, however, did

require incubation of between 12 and 24 hours, and 17.6 percent more than 24 hours, before positivity. Therefore, it remains possible that transfusion of bacterially contaminated components may occur before contamination is detected.

Since implementation of bacterial testing the risk of septic reactions to apheresis PLT transfusions has declined, but not disappeared. It is notable that coagulase-negative *Staphylococcus* was involved in the high-probability cases from screened components, which suggests that the source of contamination was the donor's skin rather than asymptomatic donor bacteremia or environmental contamination. Coagulase-negative *Staphylococci* are known to grow more slowly than coagulase-positive *Staphylococci* or Gram-negative organisms in both blood components and the BacT/ALERT system.[15,18] In each of the reaction cases, the associated culture bottles remained negative for the full shelf life of the implicated component, and sterility of the bottle was confirmed by Gram stain and culture, although in the component itself bacterial proliferation was sufficient to cause a septic reaction. Our hypothesis is that the starting concentration of organisms was sufficiently low that the bottle inoculation sample was sterile although the component was contaminated. If so, a longer waiting time before sample collection would have allowed higher concentrations of bacteria to proliferate in the component and might have helped ensure that the sample contained bacteria in sufficient concentrations (i.e., >10 CFU/mL) to guarantee a positive BacT/ALERT culture.[19] Likewise, a larger sample volume may have increased the sensitivity for detecting very low contamination levels.

Bacterial screening does not appear to have resulted in a higher component outdate rate, as might have been expected given the longer manufacturing interval and shortened shelf life at the time of distribution for most components. Our internal data indicated that for the 6-month period of July through December 2003, the number and rate of outdated components at all Red Cross locations were virtually identical when compared to those for the same period in 2004 (data not shown). We do not have data to indicate, however, whether the outdate rate at hospitals increased.

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医薬品
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識別番号・報告回数			報告日	第一報入手日 2005 年 12 月 14 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②ポリエチレングリコール処理人免疫グロブリン ③人免疫グロブリン	研究報告の公表状況	NEW ENGLAND JOURNAL of MEDICINE, 353(23), 2433-2441,2005	公表国 アメリカ		
販売名（企業名）	①献血ヴェノグロブリン-IH ヨシトミ（ベネシス） ②ヴェノグロブリン-IH（ベネシス） ③グロブリン-Wf（ベネシス）					

研究報告の概要

米国において Clostridium difficile（C. difficile）関連疾患の発生率と重症度が上昇しており、その上昇は、毒性、抗菌薬耐性、あるいはその両方が高まった C. difficile の新菌株の出現と関連している可能性が示唆されている。2000～2003 年に C. difficile 関連疾患の集団発生が起きたジョージア、イリノイ、メイン、ニュージャージー、オレゴン、ペンシルベニアの 6 州の 8 医療施設から C. difficile の分離株が計 187 株得られた。これらの分離株の特徴を、制限酵素解析（REA）、パルスフィールドゲル電気泳動、毒素タイピングによって明らかにし、その結果を 2001 年以前に採取された 6,000 株超の分離株のデータベースと比較した。ポリメラーゼ連鎖反応法を用いて、最近報告された毒素、binary toxin CDT と病原性座位を持つ遺伝子 tcdC の欠失を検出した。その結果、1 つの REA 群(BI)に属し、同じ PFGE 型(NAP1)をもつ分離株が、8 施設すべての患者の標本で同定された。5 施設では、収集した分離株の半分以上を占めた。1984 年にはじめて特定された REA 群 BI は、過去のデータベースの分離株の中にはほとんどみられなかった(14 例のみ)。過去及び最近(2001 年以降)の BI/NAP1 株はいずれも毒素型Ⅲ、binary toxin CDT 陽性で、tcdC に 18 塩基対の欠失があった。最近の BI/NAP1 株は、BI/NAP1 以外の株よりも、ガチフロキサシンとモキシフロサキシンに対する耐性が高いが、クリンダマイシンに対する耐性は両群で同等であった。最近の BI/NAP1 株はいずれもガチフロキサシンとモキシフロキサシンに耐性を示したが、過去の BI/NAP1 株で耐性を示した株はなかった。
毒素遺伝子に変異を有する C. difficile の菌株は、以前はまれであったが、フルオロキノロン系抗菌薬に対してより耐性を持つようになり、地理的に分散した C. difficile 関連疾患の集団発生の原因として出現している。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見

毒性、抗菌薬耐性、あるいはその両方が高まったC. difficileの新菌株の出現により、米国におけるC. difficile関連疾患の発生率と重症度が上昇している可能性を示唆する報告である。
C. difficileは大きさ0.5～1.9×3.0～16.9μmのグラム陽性桿菌である。万一原料尿にC. difficileが混入したとしても、除菌ろ過等の製造工程において十分に除去されると考えている。

今後の対応

本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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An Epidemic, Toxin Gene–Variant Strain of *Clostridium difficile*

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ABSTRACT

BACKGROUND

Recent reports suggest that the rate and severity of *Clostridium difficile*–associated disease in the United States are increasing and that the increase may be associated with the emergence of a new strain of *C. difficile* with increased virulence, resistance, or both.

METHODS

A total of 187 *C. difficile* isolates were collected from eight health care facilities in six states (Georgia, Illinois, Maine, New Jersey, Oregon, and Pennsylvania) in which outbreaks of *C. difficile*–associated disease had occurred between 2000 and 2003. The isolates were characterized by restriction-endonuclease analysis (REA), pulsed-field gel electrophoresis (PFGE), and toxinotyping, and the results were compared with those from a database of more than 6000 isolates obtained before 2001. The polymerase chain reaction was used to detect the recently described binary toxin CDT and a deletion in the pathogenicity locus gene, *tcdC*, that might result in increased production of toxins A and B.

RESULTS

Isolates that belonged to one REA group (BI) and had the same PFGE type (NAP1) were identified in specimens collected from patients at all eight facilities and accounted for at least half of the isolates from five facilities. REA group BI, which was first identified in 1984, was uncommon among isolates from the historic database (14 cases). Both historic and current (obtained since 2001) BI/NAP1 isolates were of toxinotype III, were positive for the binary toxin CDT, and contained an 18-bp *tcdC* deletion. Resistance to gatifloxacin and moxifloxacin was more common in current BI/NAP1 isolates than in non-BI/NAP1 isolates (100 percent vs. 42 percent, $P<0.001$), whereas the rate of resistance to clindamycin was the same in the two groups (79 percent). All of the current but none of the historic BI/NAP1 isolates were resistant to gatifloxacin and moxifloxacin ($P<0.001$).

CONCLUSIONS

A previously uncommon strain of *C. difficile* with variations in toxin genes has become more resistant to fluoroquinolones and has emerged as a cause of geographically dispersed outbreaks of *C. difficile*–associated disease.

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CLOSTRIDIUM DIFFICILE IS A GRAM-positive, anaerobic, spore-forming bacillus that can cause pseudomembranous colitis and other *C. difficile*–associated diseases. Studies during the 1970s showed that two toxins, A and B, were involved in the pathogenesis of *C. difficile*–associated disease.[1-5] Transmission occurs primarily in health care facilities, where exposure to antimicrobial drugs (the major risk factor for *C. difficile*–associated disease) and environmental contamination by *C. difficile* spores are more common.[6] Certain strains of *C. difficile* have a propensity to cause outbreaks, including multistate outbreaks in health care facilities.[7] Because these outbreak-associated strains are resistant to certain antimicrobial agents, such as clindamycin, the use of such antimicrobial agents provides these strains with a selective advantage over strains that are not associated with outbreaks. Historically low rates of severe disease and death (3 percent or less) may have led to an underestimation of the importance of *C. difficile*–associated disease as a health care–associated infection[8]; however, each case of *C. difficile*–associated disease has been estimated to result in more than $3,600 in excess health care costs, and these costs may exceed $1 billion annually in the United States.[9]

Both the rate and the severity of *C. difficile*–associated disease may be increasing in U.S. health care facilities. An analysis of data from the National Nosocomial Infections Surveillance system identified an upward slope in *C. difficile*–associated disease rates from the late 1980s through 2001.[10] Of greater concern is a reported increase of 26 percentage points between 2000 and 2001 in the proportion of patients discharged from nonfederal U.S. hospitals with *C. difficile*–associated disease listed as a diagnosis.[11]

Indications of the increased severity of *C. difficile*–associated disease include reports from the University of Pittsburgh Medical Center, where the incidence of the disease in 2000 and 2001 was nearly twice as high as in 1990 through 1999. Twenty-six patients with severe disease required colectomy, and 18 patients died.[12-14] In addition, in the past two years, the Centers for Disease Control and Prevention (CDC) has received an increased number of reports from health care facilities of cases of severe *C. difficile*–associated disease that have resulted in admissions to intensive care units, colectomies, and deaths. These reports have been confirmed by a nationwide survey of infectious-disease physicians in the Emerging Infections Network of the Infectious Diseases Society of America, which found that approximately 39 percent of respondents noted an increase in the severity of cases of *C. difficile*–associated disease in their patient population.[15]

One explanation for an increase in both the rate and the severity of *C. difficile*–associated disease could be the emergence of an epidemic strain with increased virulence, antimicrobial resistance, or both. To examine this possibility, we characterized *C. difficile* isolates obtained from health care facilities that reported outbreaks from 2001 through 2003 and compared these isolates with historic isolates (obtained before 2001) with the use of strain typing, identification of genetic determinants of newly described virulence factors, and testing for antimicrobial susceptibility.

METHODS

HEALTH CARE FACILITIES AND ISOLATES FROM PATIENTS

Isolates were collected from patients in eight health care facilities that had reported an outbreak of *C. difficile*–associated disease since 2001 to investigators at either the CDC or the Hines Veterans Affairs (VA) Hospital. These facilities were located in six states (Georgia, Illinois, Maine, New Jersey, Oregon, and Pennsylvania); all were acute care hospitals, except for one long-term care facility in Georgia that was associated with a VA hospital.[16] The isolates were obtained from patients who had received a diagnosis of *C. difficile*–associated disease on the basis of clinical history (e.g., diarrhea with recent receipt of an antimicrobial drug) and a positive clinical laboratory test for *C. difficile* toxin (e.g., cytotoxin assay or enzyme immunoassay). Isolates from current (since 2001) outbreaks were compared with isolates from a historic (pre-2001) database of more than 6000 *C. difficile* isolates maintained by Hines VA investigators. The isolates in the historic database were collected during the period from 1984 through 1990; all isolates were extensively characterized by *Hin*dIII restriction-endonuclease analysis (REA) and linked to clinical and epidemiologic data.

STRAIN TYPING

The isolates underwent REA typing and pulsed-field gel electrophoresis (PFGE), as previously described[17,18]; software from BioNumerics 3.5 (Applied Maths) was used to perform dendrographic analysis of the PFGE results. In addition, toxino-

typing was performed according to the method of Rupnik et al., with modifications.[19] Toxinotyping analyzes the restriction-fragment–length polymorphisms (RFLPs) of the genes encoding toxins A (*tcdA*) and B (*tcdB*), the surrounding regulatory genes (*tcdC* and *tcdD*), and a porin gene (*tcdE*) in a region of the *C. difficile* genome known as the pathogenicity locus (PaLoc) (Fig. 1). Because RFLP analysis of polymerase-chain-reaction (PCR) fragments A3 and B1 results in a pattern sufficient to identify most toxinotypes,[19] we limited our analysis to these two fragments.

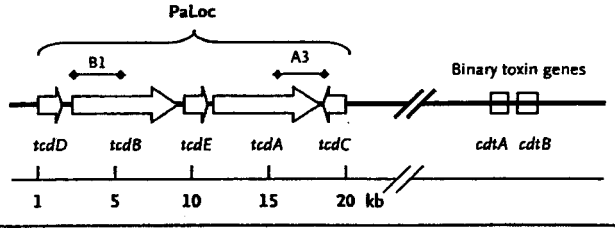


Figure 1. Major Genes in the Pathogenicity Locus (PaLoc) of *Clostridium difficile* and Relation to the Genes for Binary Toxin.

Genes *tcdA* and *tcdB* encode toxins A and B, respectively, whereas *tcdD* encodes a positive regulator of the production of toxins A and B. Gene *tcdE* encodes a protein that may be important for the release of toxin from the cell. Gene *tcdC* is a putative negative regulator of the production of toxins A and B. Genes *cdtA* and *cdtB* are located at an unknown distance from the PaLoc and encode the enzymatic and binding components, respectively, of binary toxin. B1 and A3 designate the location and relative size of the gene fragments that underwent polymerase-chain-reaction (PCR) amplification for toxinotyping.

MOLECULAR MARKERS OF POTENTIALLY INCREASED VIRULENCE

In addition to the well-characterized A and B toxins, a binary toxin has been identified in about 6 percent of clinical *C. difficile* isolates obtained in the United States and Europe.[20,21] The structure and function of this toxin (referred to as binary toxin CDT) are similar to those of other binary toxins, such as the iota toxin found in *C. perfringens*, and it is a suspected virulence factor in strains of *C. difficile* that carry the toxin.[22] We detected the *C. difficile* binary toxin gene by using PCR for *cdtB*, which is located outside the PaLoc and encodes the beta subunit of the binary toxin (Fig. 1).[20]

We also looked for deletions in *tcdC* by using PCR with the primers tcdc1 and tcdc2, which were synthesized at the CDC Core Facility on the basis of published sequences.[23] The gene *tcdC* is located within the PaLoc downstream from the genes encoding toxins A and B, and it is transcribed in the opposite direction from these genes (Fig. 1). The tcdC protein is thought to function as a negative regulator of the production of toxins A and B. Recently, multiple alleles of *tcdC* have been described that include different-sized deletions, point mutations, and in one case, a nonsense mutation, all of which would result in a truncated tcdC protein.[23,24] It has been hypothesized that mutations in *tcdC* may result in a loss of negative regulatory function, leading to increased toxin production and virulence.[23,24]

TESTING FOR ANTIMICROBIAL SUSCEPTIBILITY

Susceptibility to clindamycin and the fluoroquinolones (levofloxacin, gatifloxacin, and moxifloxacin) was determined with the use of E-test strips (AB Biodisk), and the results were interpreted according to standard criteria.[25] Specific breakpoints for the interpretation of clindamycin-susceptibility results were available from the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards).[25] However, because no breakpoints have been set by the CLSI for *C. difficile* tested against these fluoroquinolones, the CLSI breakpoints for *C. difficile* tested against trovafloxacin were used. The validity of the trovafloxacin breakpoints was confirmed by identification of two distinct subpopulations in the distribution of minimum inhibitory concentrations (MICs) for apparently susceptible isolates, as compared with resistant isolates, tested against these fluoroquinolones; these subpopulations were demarcated by the trovafloxacin breakpoints. Quality control of antimicrobial-susceptibility testing was performed during each test run with the standard strains *Enterococcus faecalis* American Type Culture Collection (ATCC) 29212, *Pseudomonas aeruginosa* ATCC 27583, *Bacteroides fragilis* ATCC 25285, and *B. thetaiotaomicron* ATCC 29741.

STATISTICAL ANALYSIS

To compare the overall resistance patterns of current epidemic and nonepidemic isolates, a total of three (determined according to the availability of isolates) epidemic-strain (case) and three nonepidemic-strain (control) isolates, as determined by REA and PFGE, were randomly selected from each health care facility. Resistance was then compared by matched case–control analysis with the use of Epi Info software (version 6.02). This method was chosen to take into account possible geographic variation in resistance and to avoid bias resulting

from outbreaks with a larger number of isolates. In contrast, we used Fisher's exact test and the StatCalc function of Epi Info software (version 6.02) to make an unmatched comparison between current and historic epidemic isolates. All P values are based on a two-tailed comparison.

RESULTS

A total of 187 isolates were obtained from the eight health care facilities in which the outbreaks occurred. In each of the facilities, a strain composed of closely related isolates was identified by both PFGE and REA. This epidemic strain accounted for 50 percent or more of the isolates from five of the eight facilities (Table 1). The epidemic strain has been identified as belonging to REA group BI and North American PFGE type 1 (NAP1). Within this strain, characterized as BI/NAP1, the isolates have been further differentiated on the basis of minor differences in the band pattern into 14 REA subtypes, designated by numbers, in which at least 90 percent of the bands are identical.[17] Similarly, several PFGE subtypes are included in the NAP1 designation. Five REA BI types (BI1 through BI5), dating back to 1984, were identified in the historic database. These represented 18 isolates obtained from 14 patients and consisted of 5 isolates of BI1 from 4 patients, 8 isolates of BI2 from 7 patients, 2 isolates of BI3 from 1 patient, 2 isolates of BI4 from 1 patient, and 1 isolate of BI5 from 1 patient.

One isolate from each of the five REA BI types in the historic database was selected for further genetic testing, along with three BI/NAP1 and three non-BI/NAP1 current isolates from each health care facility. The PFGE results and the dendrogram of these representative isolates are shown in Figure 2, along with the toxinotype, the status of binary CDT, and the status of a deletion in the *tcdC* gene. According to dendrographic analysis, 25 of 29 of the combined current and historic BI/NAP1 isolates (86 percent) were 90 percent or more related, and all were more than 80 percent related. In contrast to this close relatedness among BI/NAP1 isolates across a wide geographic area, relatively few non-BI/NAP1 isolates were more than 80 percent related. All of the BI/NAP1 isolates were of toxinotype III, were positive for binary toxin CDT, and had an 18-bp deletion in *tcdC*; these features were largely absent among non-BI/NAP1 isolates (Fig. 2). Of the 24 non-BI/NAP1 isolates, 20 (83 percent) were toxinotype 0, none of which had binary toxin CDT or the *tcdC* deletion.

Susceptibility testing was performed on the 3 current BI/NAP1 and non-BI/NAP1 isolates from each health care facility, as well as on the 14 patient BI isolates available from the historic database. Among current isolates (obtained after 2000), all BI/NAP1 and only a fraction of the non-BI/NAP1 isolates were resistant to gatifloxacin and moxifloxacin (Table 2). Although both BI/NAP1 and non-BI/NAP1 isolates were largely resistant to clindamycin and levofloxacin, the MICs of levofloxacin were higher for BI/NAP1 isolates as a group (Fig. 3). All current BI/NAP1 isolates and no historic isolates (obtained before 2001) were resistant to gatifloxacin and moxifloxacin (Table 2).

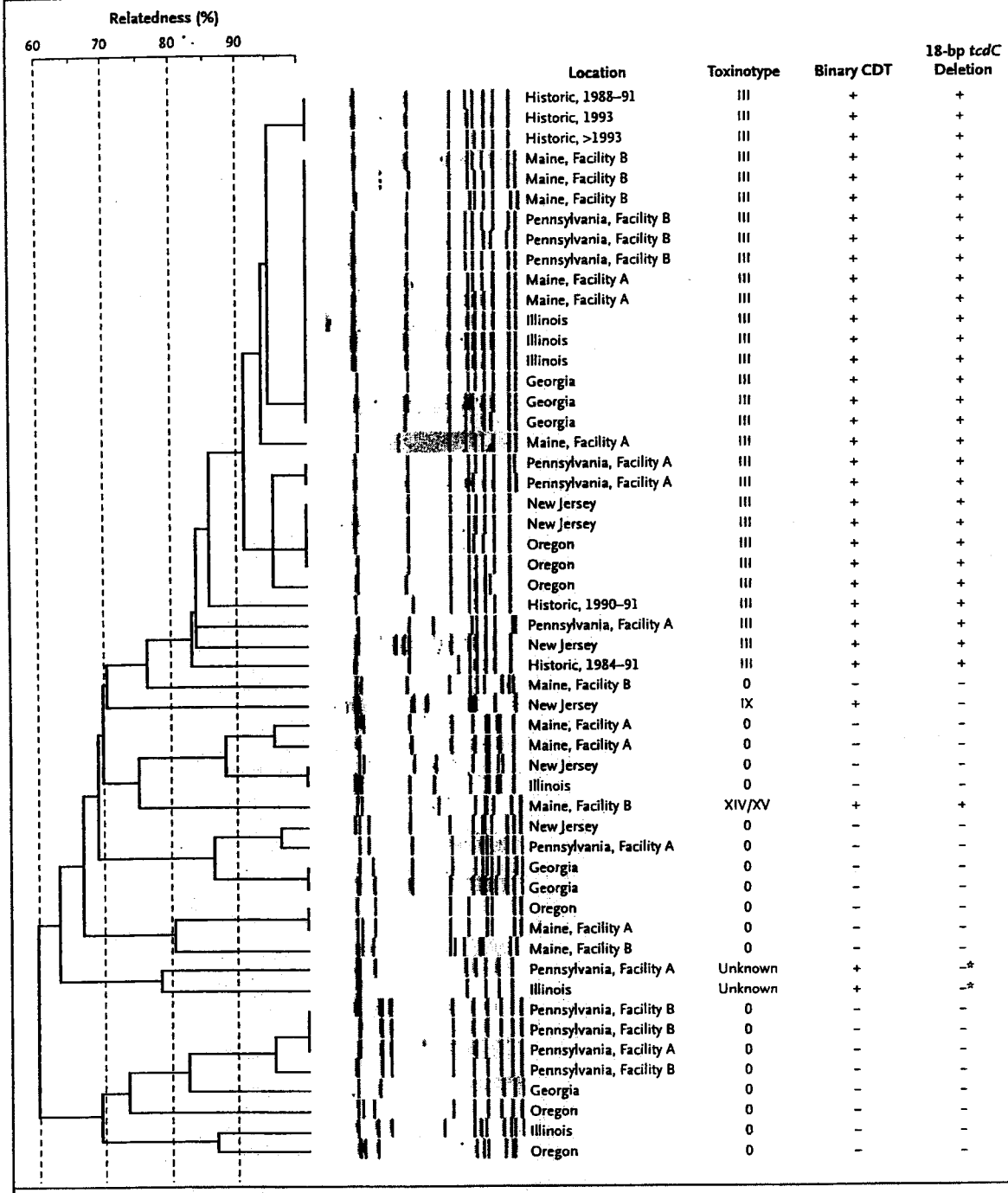
Table 1. Isolates of *Clostridium difficile* According to Health Care Facility and the Proportion of Isolates Belonging to the BI/NAP1 Strain.

Health Care Facility	Date of Onset of Outbreak	No. of Isolates Tested	BI/NAP1 Strain
			no. (%)
Georgia	Oct. 2001	46	29 (63)
Illinois	July 2003	14	6 (43)
Maine, Facility A	March 2002	13	9 (69)
Maine, Facility B	July 2003	48	30 (62)
New Jersey	June 2003	12	9 (75)
Oregon*	April 2002	30	3 (10)
Pennsylvania, Facility A	2000–2001	18	7 (39)
Pennsylvania, Facility B	Oct. 2003	6	3 (50)
Total		187	96 (51)

* Isolates were not collected until after the peak of the outbreak.

DISCUSSION

An epidemic strain of *C. difficile* has been associated with outbreaks of *C. difficile*–associated disease in eight health care facilities since 2001. This strain is the same as the strain responsible for recent outbreaks outside the United States.[26,27] It is classified by REA typing as BI and by PFGE as NAP1, and is distinct from the J strain (REA type J7/9) that was responsible for outbreaks during the period from 1989 through 1992.[28] Eighteen related isolates of the BI REA group, obtained from 14 known U.S. cases of *C. difficile*–associated disease that occurred between 1984 and 1993, were found in a database of more than 6000 isolates (representing more than 100 REA groups). According to PFGE dendrographic analysis, the majority of BI/NAP1 strain isolates



Location	Toxinotype	Binary CDT	18-bp *tcdC* Deletion
Historic, 1988–91	III	+	+
Historic, 1993	III	+	+
Historic, >1993	III	+	+
Maine, Facility B	III	+	+
Maine, Facility B	III	+	+
Maine, Facility B	III	+	+
Pennsylvania, Facility B	III	+	+
Pennsylvania, Facility B	III	+	+
Pennsylvania, Facility B	III	+	+
Maine, Facility A	III	+	+
Maine, Facility A	III	+	+
Illinois	III	+	+
Illinois	III	+	+
Illinois	III	+	+
Georgia	III	+	+
Georgia	III	+	+
Georgia	III	+	+
Maine, Facility A	III	+	+
Pennsylvania, Facility A	III	+	+
Pennsylvania, Facility A	III	+	+
New Jersey	III	+	+
New Jersey	III	+	+
Oregon	III	+	+
Oregon	III	+	+
Oregon	III	+	+
Historic, 1990–91	III	+	+
Pennsylvania, Facility A	III	+	+
New Jersey	III	+	+
Historic, 1984–91	III	+	+
Maine, Facility B	0	–	–
New Jersey	IX	+	–
Maine, Facility A	0	–	–
Maine, Facility A	0	–	–
New Jersey	0	–	–
Illinois	0	–	–
Maine, Facility B	XIV/XV	+	+
New Jersey	0	–	–
Pennsylvania, Facility A	0	–	–
Georgia	0	–	–
Georgia	0	–	–
Oregon	0	–	–
Maine, Facility A	0	–	–
Maine, Facility B	0	–	–
Pennsylvania, Facility A	Unknown	+	–*
Illinois	Unknown	+	–*
Pennsylvania, Facility B	0	–	–
Pennsylvania, Facility B	0	–	–
Pennsylvania, Facility A	0	–	–
Pennsylvania, Facility B	0	–	–
Georgia	0	–	–
Oregon	0	–	–
Illinois	0	–	–
Oregon	0	–	–

Figure 2. Pulsed-Field Gel Electrophoresis Results and Dendrographic Analysis of a Sample of BI/NAP1 and Non-BI/NAP1 Isolates from Current Outbreaks of *Clostridium difficile*–Associated Disease and of Isolates from a Historic Database.

The years listed for the historic isolates indicate years in which isolates of that type were recovered from patients, according to the database. The asterisk denotes the presence of a 39-bp deletion in *tcdC*.

Table 2. Resistance of Current BI/NAP1 *Clostridium difficile* Isolates, Current Non-BI/NAP1 Isolates, and Historic BI/NAP1 Isolates to Clindamycin and Fluoroquinolones.*

Antimicrobial Agent	Current BI/NAP1 Isolates (N=24)	Current Non-BI/NAP1 Isolates (N=24)	P Value†	Historic BI/NAP1 Isolates (N=14)	P Value‡
	no. with intermediate resistance or resistant (%)§			*no. with intermediate resistance or resistant (%)*	
Clindamycin	19 (79)	19 (79)	1.0	10 (71)	0.7
Levofloxacin	24 (100)	23 (96)	1.0	14 (100)	1.0
Gatifloxacin	24 (100)	10 (42)	<0.001	0	<0.001
Moxifloxacin	24 (100)	10 (42)	<0.001	0	<0.001

* The fluoroquinolones are levofloxacin, moxifloxacin, and gatifloxacin. Current BI/NAP1 isolates are those obtained since 2001, and historic BI/NAP1 isolates are those obtained before 2001.

† The P value is for the comparison between BI/NAP1 and non-BI/NAP1 isolates.

‡ The P value is for the comparison between current and historic BI/NAP1 isolates.

§ A minimal inhibitory concentration breakpoint of not more than 2 μg per milliliter was used for the definition of susceptibility, on the basis of the recommendations of the Clinical Laboratory Standards Institute for trovafloxacin.

(including historic BI isolates) were more than 90 percent related, and all were more than 80 percent related. Although current BI/NAP1 isolates shared with historic BI isolates the putative virulence factors of binary toxin and an 18-bp deletion in *tcdC*, the current isolates were more likely to be resistant to fluoroquinolones. Therefore, the increasing use of fluoroquinolones in U.S. health care facilities may have provided a selective advantage for this epidemic strain and promoted its widespread emergence.

The most compelling evidence of an increase in the severity of *C. difficile*–associated disease in the United States is found in the reports from Pennsylvania Facility A, where an increase in both the number of cases and the severity of the disease was noted in 2000 and 2001.[12-14] In addition, there was evidence of higher white-cell counts and more severe disease in patients infected with BI/NAP1 strains than in those infected with non-BI/NAP1 strains at the Illinois facility in our study.[29] Another report from a Connecticut hospital indicates an increase in the number of cases of severe disease necessitating colectomy during a recent outbreak associated with the BI/NAP1 strain.[30] However, reports of other outbreaks, such as the outbreak in the Georgia long-term care facility included in our study, do not suggest increased disease severity.[16] Even in the case of Pennsylvania Facility A, investigators were unable to find a significant association between the occurrence of severe *C. difficile*–associated disease and infection with the outbreak strain (P=0.23).[14] Therefore, other factors, such as underlying host susceptibility, prevailing practices of the use of antimicrobial agents or approaches to the treatment of *C. difficile*–associated disease, may have an important role in the causation of severe disease.

The importance of binary toxin CDT as a virulence factor in *C. difficile* has not been established; however, a similar toxin, iota toxin, is responsible for virulence in *C. perfringens*.[22] In previous reports, binary toxin CDT was found in only about 6 percent of *C. difficile* isolates[20,21,31]; therefore, our finding that the prevalence of this toxin is much higher in isolates from outbreaks associated with increased morbidity suggests that it could, indeed, affect the severity of *C. difficile*–associated disease. Previous studies have indicated that *C. difficile* strains with binary toxin CDT nearly always have polymorphisms in the PaLoc.[21] Binary toxin CDT has been associated with several different toxinotype patterns[31]; in our isolates, it was associated with toxinotype III, which was infrequently found in previous clinical surveys. Pseudomembranous colitis is more frequent among patients infected with *C. difficile* of toxinotype III than among patients infected with *C. difficile* of other toxinotypes, suggesting that this toxinotype is associated with increased severity of the disease.[19,21]

The importance of the 18-bp deletion in *tcdC* is currently unknown. Although *tcdC* is a proposed negative regulator of the production of toxins A and B, it is not known whether this 18-bp deletion would render a *tcdC* product nonfunctional and lead to increased production of toxins A and B.[23,24] A recent report, however, indicates that BI/NAP1 isolates in vitro do, indeed, produce toxins A and B in considerably greater quantities and at higher rates than non-BI/NAP1 isolates.[27] Nonetheless,

additional research on the effects of binary toxin CDT and of *tcdC* deletions on the severity of *C. difficile*–associated disease appears warranted.

In addition to geographic variation in disease severity, there is variation in the role of particular fluoroquinolones as risk factors in these outbreaks. The outbreak in the Georgia long-term care facility occurred after a change in the formulary from levofloxacin to a C-8-methoxy fluoroquinolone, gatifloxacin.[16] Gatifloxacin was an important risk factor for *C. difficile*–associated disease among patients, and the outbreak resolved after a formulary switch back to levofloxacin. The authors hypothesized that the higher antianaerobic activity of gatifloxacin than of levofloxacin led to a greater alteration in bowel flora and that this, combined with resistance to fluoroquinolone in the prevailing *C. difficile* strain, contributed to the outbreak.[16]

Similarly, in Pennsylvania Facility B, the outbreak started within three months after a switch in the formulary from levofloxacin to a C-8-methoxy fluoroquinolone (moxifloxacin); the preliminary results of a case–control study identify moxifloxacin as a risk factor for *C. difficile*–associated disease during the outbreak.[32] In Pennsylvania Facility A, *C. difficile*–associated disease was associated with the use of levofloxacin, clindamycin, and ceftriaxone.[13] However, a higher proportion of cases of *C. difficile*–associated disease was associated with levofloxacin (31 percent) than with clindamycin (10 percent) or ceftriaxone (7 percent).

The emergence of a previously uncommon strain of *C. difficile* that is more resistant and potentially more virulent than other strains indicates a need for inpatient health care facilities in North America to track the incidence of *C. difficile*–associated disease. Clinical outcomes of patients with *C. difficile*–associated disease should also be monitored, especially if an increase in rates is noted. If an increase in the proportion of severe cases is noted, special consideration should be given to the need for early diagnosis and treatment. Strict infection-control measures, including contact precautions, should be instituted for all patients with *C. difficile*–associated disease. In contact precautions, the patient is placed in a room alone or with another patient with *C. difficile*–associated disease, health care workers wear gloves and gowns when entering the room, and patient-care equipment (such as blood-pressure cuffs and stethoscopes) either is used only for the patient or is cleaned before it is used for another patient.[33] Enhanced environmental cleaning with dilute bleach should be used to eliminate *C. difficile* spores.[34] Because alcohol is ineffective in killing *C. difficile* spores, it is prudent for health care workers to wash their hands with soap and water, rather than with alcohol-based waterless hand sanitizers, when caring for patients with *C. difficile*–associated disease during an outbreak.[35]

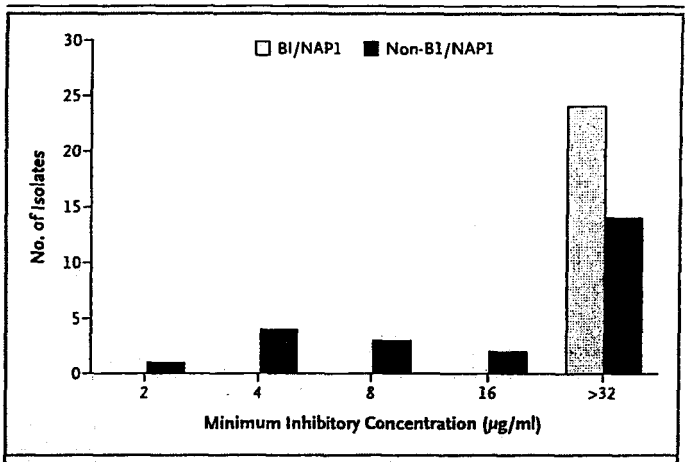


Figure 3. Distribution of Minimum Inhibitory Concentrations of Levofloxacin for Current (Obtained after 2000) BI/NAP1 and Non-BI/NAP1 *Clostridium difficile* Isolates.

Finally, an important method of controlling past outbreaks of *C. difficile*–associated disease has been restriction of the use of antimicrobial agents implicated as risk factors for the disease.[36] Whether a large-scale restriction of the use of these antimicrobial agents could slow the geographic spread of the BI/NAP1 strain is not known. Because fluoroquinolones have become a mainstay in the treatment of several common infections, a large-scale restriction of the use of these drugs would be quite difficult. However, if this epidemic strain continues to spread and to contribute to increased morbidity and mortality, it will be important either to reconsider the use of fluoroquinolones or to develop other innovative measures for controlling *C. difficile*–associated disease.

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