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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	今後の対応
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。	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 HindIII 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.

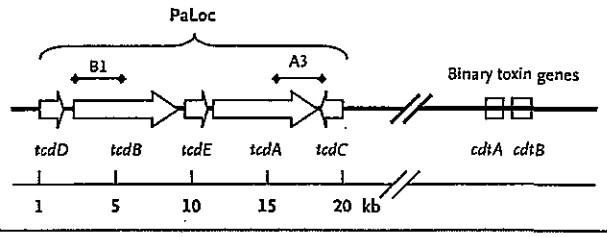


Figure 1. Major Genes in the Pathogenicity Locus (PaLoc) of *Clostridium difficile* and Relation to the Genes for Binary Toxin.

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).

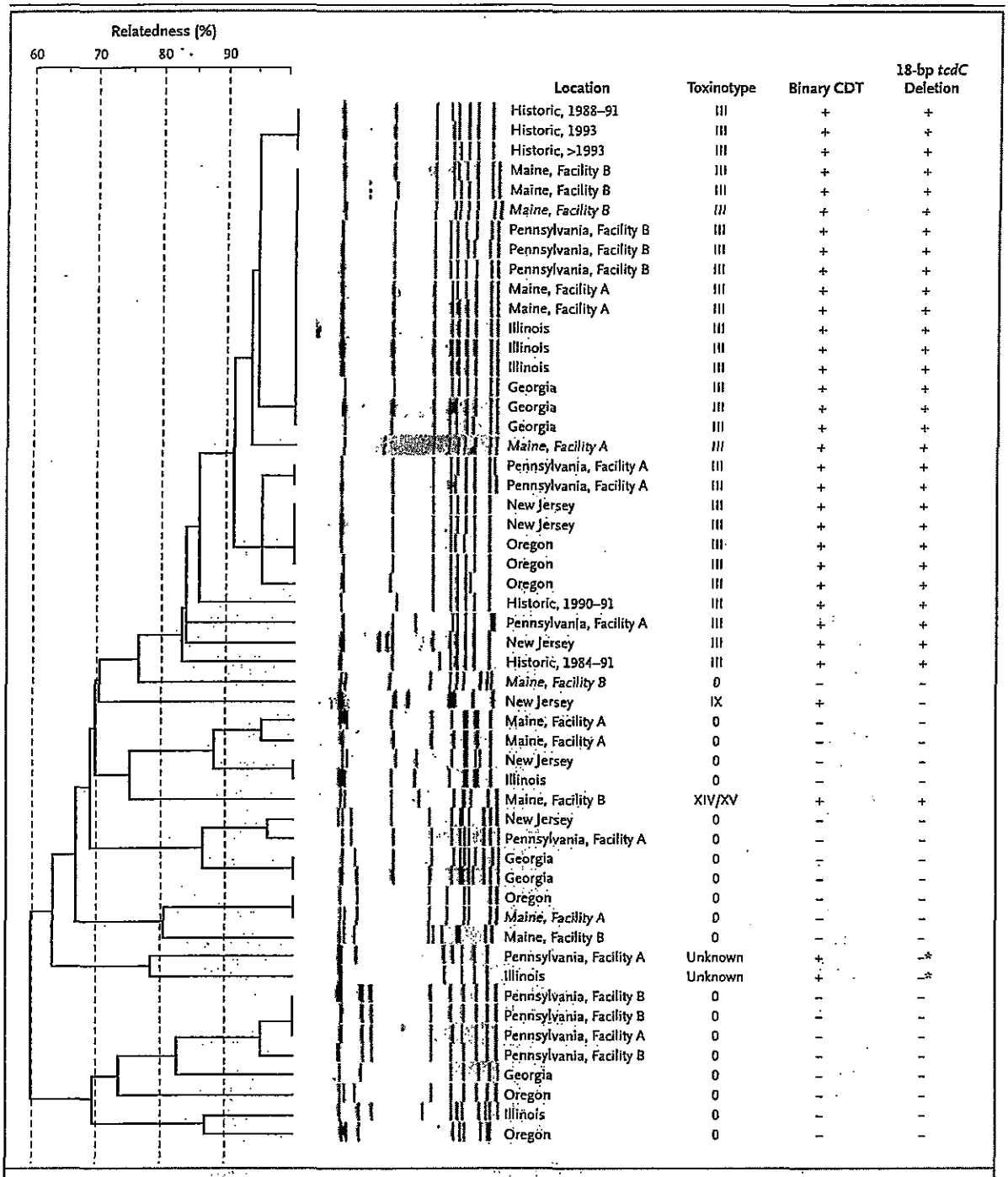
Table 1. Isolates of *Clostridium difficile* According to Health Care Facility and the Proportion of Isolates Belonging to the BI/NAP1 Strain.

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]

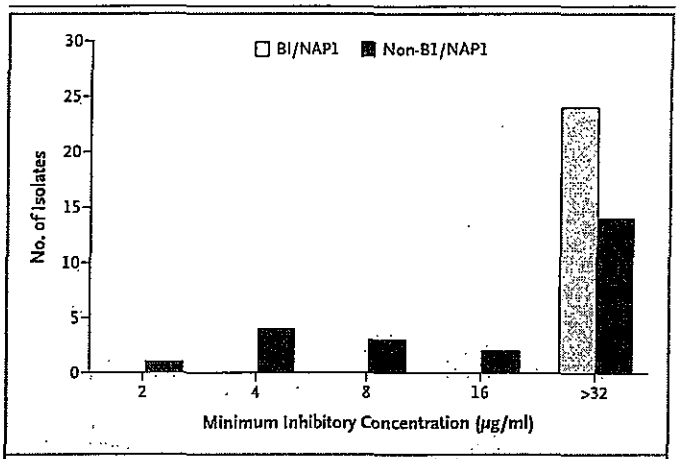


Figure 3. Distribution of Minimum Inhibitory Concentrations of Levofloxacin for Current (Obtained after 2000) BI/NAP1 and Non-BI/NAP1 *Clostridium difficile* Isolates.

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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研究報告の概要

○血小板製剤におけるリステリア菌:症例報告
背景:血小板製剤の細菌汚染低減のための取り組みから、製剤供給前の細菌検出を目的とした検査が実施されている。ヒトの病原体としては比較的まれであるが、リステリア菌は重篤な疾病を引き起こすことが多く、致死率は20%である。
症例報告:血小板供血歴の長い無症候性の58歳ヒスパニック系男性由来の血小板が、リステリア菌培養陽性となった。分離株のパルスフィールドゲル電気泳動(PFGE)パターンは、CDCのデータベースPulseNet中の他の分離株2株と一致した。公衆衛生調査からは、無症候性であったこの血小板供血者にこの2株が疫学的に関連しているという証拠は示されなかった。
結論:PFGE法によりリステリア症例のクラスターが検出されたが、臨床的意義は不明である。公衆衛生的に問題となる微生物は、衛生当局に報告される必要がある。血液製剤の安全性向上に向け、サーベイランス及び報告の改善が必要である。

報告企業の意見	今後の対応
58歳男性由来の血小板製剤から、リステリア菌が検出されたとの報告である。	日本赤十字社では、「血液製剤等に係る遡及調査ガイドライン」(平成17年3月10日付薬食発第0310009号)における「本ガイドライン対象以外の病原体の取扱い イ. 細菌」に準じ細菌感染が疑われる場合の対応を医療機関に周知している。 今後も情報の収集に努める。白血球除去の導入とともに細菌を不活化する方策についても検討を進める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Listeria monocytogenes in platelets: a case report

Ramon E. Guevara, Michael P. Tormey, Dao M. Nguyen, and Laurene Mascola

BACKGROUND: Efforts to reduce bacterial contamination in platelets (PLTs) have led to implementation of tests for bacterial detection before product release. Although relatively rare as a human pathogen, *Listeria monocytogenes* often causes serious illness and has a case-fatality rate of 20 percent.
CASE REPORT: PLTs from an asymptomatic 58-year-old Hispanic male with a long history of PLT donation were culture-positive for the presence of *L. monocytogenes*. The pulsed-field gel electrophoresis (PFGE) pattern of the isolate matched two other *L. monocytogenes* isolates in the CDC National PulseNet database. Public health investigation found no evidence that the other two isolates were epidemiologically related to the PLT donor, who remained asymptomatic.
CONCLUSION: A cluster of *listeriosis* cases was detected by PFGE but the significance is unknown. Organisms of public health significance should be reported to health departments. Better surveillance and reporting are needed in the efforts to improve blood product safety.

With successes in reducing transfusion-transmitted viruses such as human immunodeficiency virus (HIV) and hepatitis viruses,[1-4] prevention of bacterial contamination has become the next goal for improving blood product safety. Bacterial contamination of cellular blood products occurs in approximately 33 per 100,000 cellular blood product units cultured,[5,6] with prevalence rates ranging from 8 to 80 per 100,000 whole blood–derived platelet (PLT) units, 0 to 230 per 100,000 apheresis PLT units, and 0 to 3 per 100,000 red blood cell (RBC) units.[7]

Septic transfusion events due to bacterial contamination are less frequent, however, occurring in 1 per 100,000 blood product recipients.[8] Estimates of transfusion-transmitted sepsis from different studies vary[6-10] but reflect prevalence of transfusion-associated sepsis at 16 per 100,000 PLT units[11] and 0.4 per 100,000 RBC units transfused.[6,7] Possible explanations for the difference in bacterial contamination and transfusion-transmitted sepsis rates include low bacterial counts insufficient to recipient symptoms, and the frequent use of antibiotics and other recipient treatment that may mask the effects of bacteremia, including onset of sepsis.[12-15] Also, one must recognize that observation bias exists, particularly in the United States, because reports of septic reactions likely reflect only the more severe life-threatening events;[6,7] only fatalities are required for reporting to the Food and Drug Administration, and at present there is no national surveillance for such reports. Moreover, underreporting occurs because clinical personnel tend to overlook the possibility of transfusion-associated septic events because many recipients are immunosuppressed or leukopenic and therefore are susceptible to bacteremia owing to underlying disease or other causes.[5,16] Thus, because of observation bias and underreporting, rates of transfusion-transmitted sepsis may be considerably higher.

Listeria monocytogenes is a gram-positive psychrophilic (cold-loving) bacillus that causes the disease listeriosis. Although widely distributed, *L. monocytogenes* is present in low numbers in most environmental habitats and is rarely a commensal organism among humans. Nevertheless, it can cause serious sporadic and epidemic food-borne disease usually among people with lowered immune systems, particularly the elderly, pregnant

ABBREVIATIONS: ARC = American Red Cross; LAC DHS = Los Angeles County Department of Health Services; PFGE = pulsed-field gel electrophoresis; PHL = (LAC) Public Health Laboratory.

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women, neonates, patients under immunosuppressive therapy, and patients with cancer, renal disease, HIV or AIDS, or any other immunocompromising disease or condition. Common signs and symptoms of listeriosis include fever, muscle aches, nausea, diarrhea, headache, stiff neck, confusion, loss of balance, convulsions, premature birth, and stillbirth. Unlike reports for more common food-borne diseases like salmonellosis and campylobacteriosis, reports of listeriosis usually describe serious illness, like sepsis or meningitis, causing hospitalization and sometimes death. *L. monocytogenes* causes approximately 2500 illnesses, 2300 hospitalizations, and 500 deaths in the United States per year and has a case-fatality rate of 20 percent.[17] Risk foods include raw milk, raw-milk products like soft cheese, raw fruits and vegetables, raw or undercooked meats and seafood, and ready-to-eat foods like bagged salads, hot dogs, and deli meats. Because the incubation period of *L. monocytogenes* ranges from 3 to 70 days with a median of 3 weeks, identifying the source of infection is often very difficult.

We report a case of PLT contamination detected before product release. Since late February 2004, the American Red Cross (ARC) of Southern California has tested all PLTs for bacteria. *L. monocytogenes* has not been previously reported as a PLT contaminant. The investigation of this case demonstrates how an organism of public health importance has potential health implications for the donor and recipients and why collaborating with the health department is important.

CASE REPORT

In October 2004, the ARC of Southern California reported a repeat-positive bacterial culture result from an apheresis PLT product that was subsequently split into 2 units. The contaminating organism was identified as *L. monocytogenes*. The contaminated PLTs were destroyed and not released for transfusion. The donor made four apheresis PLT donations in the subsequent month at a hospital-based blood bank and these all tested negative for bacterial contamination.

MATERIALS AND METHODS

To test for bacterial contamination, the ARC of Southern California used an automated system (BacT/ALERT 3D, bioMérieux, Durham, NC) with each aerobic bottle (BacT/ALERT BPA) inoculated with 4.0 mL of PLT product. Sampling devices included a sampling kit (SampLok, Innovation Technology Licensing, Canberra, Australia) and a sterile tubing welder (Terumo, Tokyo, Japan). Sampling was carried out 24 hours after donation in a laminar flow hood. A contracted microbiology reference laboratory performed Gram stain and species identification. For the described donor case, the mother bag and two daughter bags were sampled with BacT/ALERT BPA aerobic bottles. In addition, with the Terumo sterile tubing welder and plastic transfer packs, 50 to 100 mL from each daughter bag was taken for microbiologic testing.

To test for bacterial contamination in the later apheresis PLT donations by the described case, the hospital blood bank used the classic bioMérieux BacT/ALERT automated system. Each BacT/ALERT BPA aerobic bottle was inoculated with 4.0 mL of PLT product and sampling devices included a Terumo sterile connecting device with a Charter Medical (Winston-Salem, NC) plasma-fluid transfer set. Sampling was performed 24 to 36 hours after donation and was not in a laminar flow hood.

The Los Angeles County Department of Health Services (LAC DHS) performs surveillance on listeriosis by requiring all diagnostic laboratories and health-care providers licensed in LAC to report cases and submit *L. monocytogenes* isolates to the LAC Public Health Laboratory (PHL). The PHL confirms identification of *L. monocytogenes* and uses *Asc*I and *Apa*I enzymes to analyze isolates by pulsed-field gel electrophoresis (PFGE).[18] The PHL submits results to the Centers for Disease Control and Prevention (CDC) for comparison to a US national database called PulseNet.[19] When an isolate from LAC has a similar if not indistinguishable PFGE pattern with any other isolate in the national database and the collection dates of the isolates occur within 120 days of each other, CDC informs LAC DHS. Since 1985, LAC DHS has investigated, collected, and analyzed listeriosis case data for disease trends and outbreak detection.

Case investigation normally consists of collaborations with hospital infection control practitioners, medical records offices, and LAC DHS public health nurses to collect data on clinical presentation and predisposing factors. Ultimately the listeriosis surveillance epidemiologist at LAC DHS interviews cases or available case relatives for medical, food, and travel history.

The occurrence of at least two listeriosis cases with the same source of infection confirms an outbreak. Since 1999, LAC DHS has used PFGE to assist in the identification of outbreaks,[20] particularly when PFGE patterns are rare and occur suddenly in more numbers. When investigating situations that may become outbreaks, LAC DHS investigators develop hypothesis-generating questionnaires to gather more details of possible sources of infection. The hypothesis-generating questionnaire for the investigation described in this report consisted of questions on history of blood transfusion, dental work, excavation around the home, travel, and food history specifics such as purchase location, dates, frequency of consumption, and food product brands and names. To improve case detection, LAC DHS alerted all infection control practitioners in LAC of the PLT findings and requested immediate reports of listeriosis cases not yet reported.

RESULTS

In October 2004, the ARC of Southern California called LAC DHS to ask if blood banks were required to report blood products testing positive for the presence of *L. monocytogenes* even if the donor was asymptomatic and had no history of illness. LAC DHS verified that such instances needed to be reported and learned from ARC that two PLT bags from a single apheresis donation by a 58-year-old Hispanic male with no signs or history of illness tested positive for the presence of *L. monocytogenes* (Case 1). Bacterial contamination was detected at 21.4 hours of incubation. The two daughter bags from the plateletpheresis collection were quarantined, and although each tested negative for the presence of bacteria after 5 days of incubation of the BacT/ALERT BPA aerobic bottles, one of the 50 to 100 mL samples from the daughter bags grew *L. monocytogenes*. This was the first time the ARC of Southern California had identified *L. monocytogenes* in a blood product.

In mid-November 2004, CDC informed LAC DHS that two subsequent cases, one in LAC (Case 2) and one in Colorado (Case 3), shared the same PFGE pattern defined by the *Asc*I and *Apa*I enzymes. Including these three incidents, the pattern appeared only eight times (0.19%) in the national database of 4167 isolates analyzed by both enzymes. LAC had two other isolates with this pattern, one occurring in 2003 (Case 4) and one in 1999. Despite a health alert to all infection control practitioners in LAC, no further listeriosis cases with this PFGE pattern were reported.

Case investigation focused on the two 2004 LAC cases but extended to Case 3 and Case 4 (Table 1). The PLT donor (Case 1) had no risk factors for listeriosis and ate only a few risk foods (cottage cheese, Gouda cheese, mozzarella cheese). He had no symptoms of illness before or during his *Listeria*-positive PLT donation. Since 2001, he donated PLTs only and had made 12 donations since the ARC started testing PLTs for bacteria. Because previous donations were culture-negative and he was asymptomatic, the donor was allowed to continue donation. He was not recultured, but he subsequently made four separate apheresis PLT donations at a hospital blood bank during October and November and these were all negative for the presence of bacterial contamination. In Case 2, a 58-year-old Hispanic woman developed symptoms and died around the same time as the PLT donation of Case 1. She died the day after hospital discharge with the cause of death listed as breast cancer. Although her surviving relatives recalled her getting a blood transfusion for anemia 2 months before her illness onset, hospital and hospice records documented only the anemia and not the transfusion. This case had multiple risk factors including breast cancer with metastases to liver, bone, lung, and brain; recent chemotherapy and steroid medication; and recent antacid use. The patient of Case 2 also ate several risk foods, such as Mexican-style fresh cheese, soft cheese, deli meats, and raw seafood. The only common food to Cases 1 and 2 was mozzarella cheese. The distance between the case residences, different brands of mozzarella, and lack of further cases with history of mozzarella consumption made the cheese an unlikely common source. Including information from Case 3, a 74-year-old woman with listeriosis in October 2004, and Case 4, a 59-year-old man from LAC with listeriosis and metastatic adenocarcinoma in August 2003, no useful epidemiologic connections could be made among these four cases other than the PFGE pattern.

TABLE 1. Listeriosis cases with indistinguishable PFGE pattern—United States, 2003 to 2004

Case	Age (years)	Sex	Location	Specimen collection date
1	58	Male	LAC, CA	September 27, 2004
2	58	Female	LAC, CA	October 1, 2004
3	74	Female	Colorado	October 19, 2004
4	59	Male	LAC, CA	August 14, 2003

DISCUSSION

The most unusual characteristic of this listeriosis investigation was that the PLT donor was asymptomatic with no history of recent illness. Listeriosis cases with bacteremia normally have fever or at least some other symptom. In a review of 1036 listeriosis cases in LAC, only one other nonpregnant adult case had bacteremia and was asymptomatic. The best explanation the authors have regarding the PLT donor is transient bacteremia. Bacterial contamination of blood products has been ascribed to transient bacteremia in the past.[6,7,21] Because CDC found two other cases with the same rare PFGE pattern around the same time frame, the reference laboratory of the ARC of Southern California grew cultures from two of five samples taken at different times, and the PHL confirmed *L. monocytogenes*, environmental contamination, false-positive laboratory results, and skin contamination were thought to be less likely explanations. Furthermore, the lack of predisposing medical conditions in Case 1 probably contributed to his lack of symptoms as the other cases had risk factors for listeriosis.

Listeriosis caused by transfusion has not yet been reported, at least in the literature. In 1998, a case report from Trinidad described a premature infant returning to the hospital with septicemia and meningitis due to *L. monocytogenes* approximately 4 days after receiving a whole-blood transfusion.[22] Transfusion-transmitted listeriosis, however, was not definite because the mother was not

ruled out as the source of infection and the whole blood used for transfusion was not cultured. Over 3 years of active surveillance of 60 to 70 percent of blood banks in the United States, the Assessment of the Frequency of Blood Component Bacterial Contamination Associated with Transfusion Reaction Study (BaCon) found no *L. monocytogenes* bacteremia cases; however, the BaCon study defined bacteremia cases as blood product recipients with signs or symptoms occurring within 4 hours of transfusion and required culture confirmation in both the blood component and the patient, reducing sensitivity for reported cases.[8]

The LAC case of the contaminated PLTs was detected because the ARC of Southern California began testing apheresis PLTs for bacterial contamination in February 2004. This was in anticipation of the standard the AABB adopted on March 1, 2004, for blood banks and transfusion services to have methods to detect and limit bacterial contamination in all PLT components.[23] Whole blood–derived PLTs, RBCs, and plasma are not typically cultured to detect bacterial contamination. Other proposed methods to reduce bacterial contamination in blood products include improving donor screening, better skin disinfection, pathogen inactivation by chemical or photochemical methods, and testing RBC units during the second week of storage.[5-7,24-27] The overall benefits, costs, and risks need to be carefully considered before implementing any method to improve blood safety.

This investigation revealed that in conducting surveillance for listeriosis, blood-related issues need more scrutiny. Although iron overload has been established as a risk factor for listeriosis,[28,29] measurement of this suffers from diagnostic bias because testing really only happens for patients with repeated transfusions for severe or chronic anemias such as thalassemia major, myelodysplasia (including sideroblastic anemia), moderate aplastic anemia, and Diamond-Blackfan anemia.[30] Given published evidence of iron increasing the growth and lethality of *L. monocytogenes*,[31-33] researchers should measure recent history of anemia, blood transfusions, and iron supplements as risk factors for listeriosis.

The critical event for this case report was the ARC reporting to the health department. Reporting by blood banks and health-care facilities is necessary to determine the risks and boundaries of possible outbreaks, particularly if contaminated products are released for transfusion. Although the contaminated products were not released in this case, the donation history of the PLT donor became important to determine whether he previously donated RBC units that might have caused other cases. Health departments in areas with little or no experience with listeriosis might not have required notification of this case. Because PLTs and other blood components found to be positive for the presence of reportable organisms require notification of public health authorities, health departments at all levels of government should ensure that reporting requirements are clear for various reporting sources, especially blood banks, and in other settings in which there are new guidelines or standards. A recent CDC survey of clinicians demonstrated low awareness of the new AABB standard for bacterial testing of PLTs and of transfusion-transmitted infectious disease risks by bacteria-contaminated PLTs.[34] This finding, plus the inclusion of several statements to save culture isolates and notify appropriate state and local health departments in the AABB February 2005 guidelines for recognizing and managing transfusion reactions,[35] reflect the fact that better surveillance and reporting are needed in the efforts to improve blood product safety.

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