Ewingella americana: Recurrent Pseudobacteremia from a Persistent Environmental Reservoir

MICHAEL M. McNEIL,^{1*} BARRY J. DAVIS,¹ STEVEN L. SOLOMON,¹ ROGER L. ANDERSON,¹ STANFORD T. SHULMAN,² SHERRY GARDNER,² KATHY KABAT,² AND WILLIAM J. MARTONE¹

Hospital Infections Program, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333,¹ and Division of Infectious Diseases, Department of Pediatrics, The Children's Memorial Hospital, Northwestern University Medical School, Chicago, Illinois 60614²

Received 19 August 1986/Accepted 3 December 1986

From September 1981 through April 1984, 20 patients at one hospital were identified with *Ewingella americana* pseudobacteremia. Case-control studies demonstrated an association between having a positive blood culture for *E. americana* and having blood for culture obtained simultaneously with blood obtained for coagulation studies (15 of 19 case patients versus 4 of 38 controls; $P = 4.5 \times 10^{-7}$). Review of blood-drawing procedures showed that (i) blood for coagulation studies and culture was drawn with the same syringe, and (ii) coagulation tubes were filled before blood culture tubes. Some phlebotomists were not using new sterile needles to inoculate blood culture bottles. Collection tubes for coagulation studies were prepared in the hospital, and *E. americana* was isolated from all 52 unused coagulation tubes tested. Solutions prepared in the hospital may constitute a persistent inanimate environmental reservoir for this uncommon microorganism. Pseudobacteremia can result in unnecessary antimicrobial therapy for some patients, incurring the risks of adverse drug reactions, selection of drug-resistant bacteria, and increased health care costs.

Since *Ewingella americana* was first described in 1983, it has rarely been associated with patient illness (1). In one report, patients developed bacteremia after cardiovascular or peripheral vascular surgery related to contamination of an ice bath used to cool syringes for cardiac output studies (8). This report describes our investigation of an outbreak of pseudobacteremia with *E. americana* traced to cross contamination from nonsterile citrated blood collection tubes.

MATERIALS AND METHODS

Background. From September 1981 through April 1984, the microbiology laboratory of a 265-bed pediatric referral hospital, isolated an unusual gram-negative bacillus from the blood cultures of 11 patients. This microorganism was identified as a rare biotype of *Enterobacter agglomerans* with the Enterotube II system (Roche Diagnostics, Div. Hoffman-La Roche Inc., Nutley, N.J.). The reference diagnostic laboratory sent 13 isolates from these 11 patients through the Illinois State Public Health Laboratory to the Centers for Disease Control for further identification. The 13 isolates were all determined to be *E. americana*. The hospital initiated an investigation to determine the clinical and epidemiologic significance of these positive blood cultures and invited the Centers for Disease Control to participate.

Epidemiologic investigation. Case definition. A case patient was defined as any patient with a blood culture positive for *E. americana*.

Case ascertainment. Because the Enterotube II system had identified the 13 confirmed *Ewingella* isolates as *E. agglomerans*, we reviewed all hospital microbiology laboratory reports since 21 July 1980 to identify those isolates identified as *E. agglomerans* that had (i) the same Enterotube II system code number, and (ii) the same antimicrobial susceptibility pattern (resistance only to cephalothin) as that of the

13 known *E. americana* isolates. Using these criteria, we identified an additional nine isolates from nine different patients, which were confirmed as *E. americana* at the Centers for Disease Control reference laboratory.

Review of case patients. We reviewed the medical records of all case patients and abstracted information on age, sex, hospital service, length of stay, underlying illnesses, indications for blood cultures, exposure to invasive lines and therapies, receipt of antibiotics, clinical and laboratory data, and outcome of hospitalization.

Case-control study. We conducted a case-control study to identify potential risk factors for having a positive blood culture and possible reservoirs for these microorganisms. Two controls were matched to each case patient by ward and date of positive blood culture using the blood culture logbook to identify two patients (i) whose blood cultures were received in the laboratory immediately before and after the blood culture of the case patient, and (ii) who were located in the same area or unit of the hospital as the case patient when their blood was drawn for culture. The charts of control patients were reviewed for the same details as were those of case patients.

Procedure review. We interviewed hospital personnel about blood-drawing practices and observed blood being drawn for culture. We observed laboratory procedures for handling blood cultures and reviewed practices used for other kinds of blood tests, especially coagulation studies, including how specimens were obtained and how blood collection tubes were prepared and stored. We also interviewed personnel in the engineering department concerning water sources and water treatment facilities in the hospital.

Statistical methods. Statistical analyses were done by the chi-square and one-tailed Fisher's exact tests (7, 10).

Laboratory investigations. Method of isolation from tubes. Unused coagulation tubes and unused screw-top tubes to which no anticoagulant had been added were cultured aseptically. The citric acid and sodium citrate used to prepare the anticoagulant solution were cultured separately. However,

* Corresponding author.

FIG. 1. *E. americana* cases from September 1980 to April 1984.

the anticoagulant solution had most recently been prepared approximately 4 months before the investigation.

Method of identification. Standard biochemical methods were used to identify isolates of *E. americana* (1).

Antimicrobial susceptibility testing. The susceptibility patterns of all the *E. americana* isolates of the case patients and 15 of the 52 coagulation tube isolates were determined by disk susceptibility testing (5); in addition, MICs were determined for all of these isolates (6).

Environmental investigation. We cultured samples of incoming city water, water from the central still, and distilled deionized water from the chemistry laboratory. We cultured the resin cores of the deionization unit in the chemistry laboratory and distilled deionized water recovered when this unit was dismantled. We also tested distilled deionized water collected from the chemistry laboratory in August 1984, 2 months after the unit's last service, and again in April 1985.

RESULTS

Epidemiologic investigation. Case ascertainment. Twenty case patients were identified. They all had their first positive culture for *E. americana* from September 1981 to April 1984 (the epidemic period; Fig. 1). At the time their blood was drawn for positive cultures, 18 case patients were located in either the emergency room (ER; 6 patients) or the intensive care unit (12 patients); 2 case patients were located in other wards. Two case patients each had two blood cultures positive for *E. americana*. Ten case patients were male; their mean age was 4.4 years (range, 3 h to 14 years).

The mean duration of stay in the hospital of case patients was 29 days (range, 3 to 82 days; Table 1). The mean duration from the time of admission to time of first blood culture positive for *E. americana* was 7.3 days (range, 0 to 47 days). Only one case patient did not receive any antibiotic therapy. After their positive blood cultures, 14 case patients received antibiotics appropriate for the treatment of gram-negative bacteremia. However, it was not possible in all cases to determine based on the hospital record whether this antibiotic therapy was initiated or altered specifically to cover *E. americana*. No patient had clinical disease specifically attributable to *E. americana* sepsis. Four case patients died of their underlying disease.

Case-control study. The case-control study showed that case patients and controls did not differ significantly in their history of exposure to invasive lines and therapies (Table 2). In the intensive care unit and ER, blood cultures were often drawn simultaneously with blood for other tests, especially blood gas analyses and coagulation profiles. There was no significant difference between case patients and controls in

the timing of blood cultures and blood for blood gas analysis. However, having a positive blood culture was statistically associated with having had blood for culture obtained at the same time blood had been drawn for a coagulation profile (15 of 19 case patients versus 4 of 38 controls; $P = 4.5 \times 10^{-7}$, Fisher's exact test, one-tailed; Table 2).

Procedure review. Interviews with nursing personnel showed that some phlebotomists who obtained blood for culture and coagulation tests in the same syringe filled the nonsterile coagulation tube first to ensure that the blood did not clot. We also found that drawing blood for culture and coagulation tests simultaneously was unusual outside the ER and intensive care unit and that the standard practice for obtaining blood cultures of attaching a new sterile needle before inoculating the blood culture media (11) had not been uniformly observed. Until late April 1984, 5-ml screw-cap plastic tubes containing a citrate solution had been used in the ER and intensive care unit to collect blood for coagulation studies; these tubes had been supplied by the chemistry laboratory of the hospital. The chemistry laboratory had prepared batches of these tubes with a stock citrate solution consisting of crystalline sodium citrate and citric acid dissolved in distilled deionized water. This stock citrate solution was prepared in 500-ml amounts approximately three times per year and kept refrigerated. Laboratory personnel prepared blood collection tubes as needed by dispensing 0.3-ml amounts of citrate solution into the individual plastic tubes. On the wards, these tubes were kept refrigerated until used. In April 1984, the hospital began to purchase commercially available VACUTAINER tubes (Becton Dickinson Vacutainer Systems, Rutherford, N.J.), which are labeled as having a sterile interior and may be stored without refrigeration. This change was made because the VACUTAINER tubes were considered to be more convenient and cost effective and not because contamination of the hospital-prepared tubes was suspected.

Laboratory investigation. Recovery from tubes. Coagulation tubes prepared before April 1984 were recovered from refrigerators on wards and clinics in the hospital. *E. americana* was recovered in pure culture from all 52 coagulation tubes examined. Quantitative assay performed on a subset of these tubes showed levels of 10^2 to 10^4 CFU of *E. americana* per ml. Dry coagulation tubes to which no citrate solution had been added were not contaminated. No *E. americana* could be isolated from distilled deionized water, the ingredients of the citrate anticoagulant solution, or the deionized resins.

Susceptibility testing. All isolates from patients and coagulation tubes shared the same antimicrobial susceptibility pattern when tested at 35°C (i.e., resistance only to cephalothin).

DISCUSSION

Pseudobacteremia, the occurrence of false-positive blood cultures, has resulted from the introduction of contaminating organisms either at the time the blood specimen is drawn or during its laboratory processing (3, 9). When nonsterile coagulation tubes have been implicated in hospital-based outbreaks of pseudobacteremia, incorrect blood-drawing techniques similar to those identified in this investigation were noted (2, 9, 12; B. D. Cookson, S. Mehtar, and G. Sadler, Letter, Lancet ii:1276-1277, 1982; K. Whale, Letter, Lancet i:830, 1983). In this outbreak, the coagulation tube was filled first, and contact with the nonsterile interior of the tube apparently contaminated the syringe, which led to cross contamination of the blood culture (4).

Although *E. americana* contamination was traced only as

TABLE 1. Characteristics of patients in case-control study of *E. americana* pseudobacteremia, September 1981 to April 1984

Group and no. of patients	No. of patients (%)					Mean no. of days of hospitalization:	
	Sex		Admitted from:		Previous admissions to same hospital	Until 27 May 1984	Before positive culture
	Male	Female	Home	Another hospital			
A							
Case patients (<i>n</i> = 19)	9	10	9 (47)	10 (53)	5 (26)		
Controls (<i>n</i> = 38)	25	13	33 (87)	5 (13)	18 (47)		
<i>P</i> value ^a	NS		0.001		NS		
B							
Case patients (<i>n</i> = 17) ^b						29	7 ^c
Controls (<i>n</i> = 32) ^b						42	16 ^d
<i>P</i> value ^e						NS	NS

^a Chi-square, 1 df. NS, Not significant.^b Excludes patients seen in ER but not admitted.^c Represents the time from admission to the time of the first positive culture for *E. americana*.^d Represents the time from admission to the time of matched blood culture.^e *t* test; comparison of two independent means. NS, Not significant.

far as the coagulation tubes and the primary reservoir in the hospital was not defined, the most likely source of *E. americana* was distilled deionized water used in the hospital chemistry laboratory to prepare these tubes.

Studies conducted in our laboratory may explain the ability of the epidemic microorganism to persist unnoticed for so long in the hospital environment. Growth studies using the hospital's distilled deionized water and citrate solution prepared from it suggest that *E. americana* may be ideally suited to survival in citrate solutions (R. L. Anderson, B. W. Holland, B. J. Davis, M. M. McNeil, and M. S. Favero, submitted for publication). Preferential growth of the organism at 4°C has also been demonstrated (Anderson et al., submitted). Therefore, had low-level, intermittent contamination of the hospital water occurred with *E. americana*, subsequent storage conditions might explain our finding it as the sole contaminant of the coagulation tubes.

We were unable to isolate *E. americana* from samples of distilled deionized water collected on four occasions over an 11-month period. However, we collected these samples in the summer and spring, long after the water used to prepare the contaminated coagulation tubes would have been available. Although our investigation failed to uncover the origi-

nal source of the epidemic strain, it established a persistent inanimate environmental reservoir for this uncommon microorganism, which had gone unnoticed in the hospital for 32 months and could have resulted in unnecessary antimicrobial therapy for some patients.

ACKNOWLEDGMENTS

We are indebted to Betty W. Holland and Bertha C. Hill for laboratory assistance and Irene Cason-Tucker for preparation of the manuscript.

LITERATURE CITED

1. Grimont, P. A. D., J. J. Farmer III, F. Grimont, M. A. Asbury, D. J. Brenner, and C. Deval. 1983. *Ewingella americana* gen. nov., sp. nov. A new *Enterobacteriaceae* isolated from clinical specimens. Ann. Microbiol. (Paris) **134A**:39-52.
2. Hoffman, P. C., P. M. Arnow, D. A. Goldmann, P. L. Parrott, W. E. Stamm, and J. E. McGowan, Jr. 1976. False-positive blood cultures. Association with nonsterile blood collection tubes. J. Am. Med. Assoc. **236**:2073-2075.
3. Maki, D. G. 1980. Through a glass darkly. Nosocomial pseudoeidemics and pseudobacteremias. Arch. Intern. Med. **140**:26-28.
4. McNeil, M. M., B. J. Davis, R. L. Anderson, W. J. Martone, and S. L. Solomon. 1985. Mechanism of cross-contamination of blood culture bottles in outbreaks of pseudobacteremia associated with nonsterile blood collection tubes. J. Clin. Microbiol. **22**:23-25.
5. National Committee for Clinical Laboratory Standards. 1982. Performance standards for antimicrobial disc susceptibility tests, vol. 2, no. 2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
6. National Committee for Clinical Laboratory Standards. 1983. Standard methods for dilution antimicrobial tests for bacteria which grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
7. Ostle, B. 1969. Statistics in research, 2nd ed. Iowa State University Press, Ames.
8. Pien, F. D., and A. E. Bruce. 1986. Nosocomial *Ewingella americana* bacteremia in an intensive care unit. Arch. Intern. Med. **146**:111-112.
9. Semel, D. J., G. M. Trenholme, A. A. Harris, J. E. Jupa, and S. Levin. 1978. *Pseudomonas maltophilia* pseudosepticemia. Am. J. Med. **64**:403-406.
10. Snedecor, G. W., and W. G. Cochran. 1980. Statistical methods, 7th ed. Iowa State University Press, Ames.
11. Stratton, C. W. 1981. Endemic staphylococcal pseudobacteremia. Infect. Control (Thorofare) **2**:251-252.
12. Willson, P. A., D. N. Petts, and S. L. Baker. 1981. An outbreak of pseudobacteremia. Br. Med. J. **283**:866.

TABLE 2. Case-control study risk factors for *E. americana* pseudobacteremia

Risk factor	No. of case patients (<i>n</i> = 19)	No. of controls (<i>n</i> = 38)	<i>P</i> value
Arterial line	9	15	NS ^a
Central line	12	13	0.04 ^a
Endotracheal tube	10	18	NS ^a
Intravenous therapy	13	24	NS ^a
Urinary catheter	8	12	NS ^a
Nasogastric tube	11	17	NS ^a
Intracranial pressure monitor	3	2	NS ^b
Respiratory therapy	11	20	NS ^a
Surgery	8	14	NS ^a
Transfusion	10	18	NS ^a
Other blood test	18	24	8.3 × 10 ^{-3b}
Coagulation or arterial blood gas	16	11	8.3 × 10 ^{-5b}
Arterial blood gas	5	8	NS ^b
Coagulation	15	4	4.5 × 10 ^{-7b}

^a Chi-square, 1 df. NS, Not significant.^b Fisher's exact test (one-tailed). NS, Not significant.