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Bacillus Species Pseudobacteremia Traced to Contaminated Gloves Used in Collection of Blood from Patients with Acquired Immunodeficiency Syndrome

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Ten nonpathogenic *Bacillus* isolates were obtained from blood cultures collected over a 2-year period. Eight of these isolates were from patients with acquired immunodeficiency syndrome, and seven were recovered from blood cultures obtained in outpatient clinics. Five cases occurred during a 5-month period. These five cases were clinically evaluated, and the *Bacillus* isolates were characterized. The same *Bacillus* species was isolated from nonsterile gloves from the same lot worn by phlebotomists for blood collection in the outpatient clinics during this period, implicating the gloves as the cause of this pseudoepidemic. Awareness of the nonsterile nature of gloves used by laboratory personnel should be considered in the evaluation of *Bacillus* spp. in blood cultures.

Pseudobacteremia is suspected when there is a cluster of positive blood cultures with a single unusual species or when the microbiological findings do not correlate with the clinical findings (14). A variety of factors can cause pseudobacteremia, including (i) the environment in which the blood is collected (15), (ii) contaminated disinfectants (1), (iii) contaminated specimen collection tubes (10), (iv) contaminated blood culture media (16), and (v) contamination during sampling in automated blood culture detection systems (3, 6, 9, 13). It has been demonstrated that 11% of suspected nosocomial epidemics are in fact "pseudooutbreaks" due to the aforementioned factors (4, 19). This paper reports a pseudoepidemic of *Bacillus* bacteremia in patients with acquired immunodeficiency syndrome (AIDS) which was traced to the gloves used by the phlebotomists.

Background. From 1 July 1985 to 1 July 1987, a total of 24,499 blood cultures were received by the Microbiology Section of the Clinical Laboratories at the University of California Medical Center; 1,494 originated from the outpatient clinics located in the Ambulatory Care Center directly across the street. Most blood culture collections for the clinics are performed in the Outpatient Blood Drawing Station (OPBDS) on the first floor of the Ambulatory Care Center. The positivity rate for *Bacillus* isolation from blood cultures in the outpatient clinics was 0.47% (7 of 1,494). For inpatient blood culture collections, the rate was 0.01% (3 of 23,005) ($P = 0.01$). Of the 10 *Bacillus* isolates recovered, 8 were from AIDS patients (Table 1). Of the seven isolates collected in the OPBDS, two (Table 1, from patients 3 and 4) were recovered during a 1-week period and five (Table 1, from patients 5 to 9) were recovered during a 5-month period.

The latter five isolates were obtained from febrile AIDS patients (Table 1, patients 5 to 9). Although all five patients were admitted to the hospital, patients 5 and 9 were admitted solely because of the positive blood cultures. Patients 5 and 6 were treated for gram-negative sepsis and became afebrile on therapy. Patients 7, 8, and 9 were diagnosed as having *Pneumocystis carinii* pneumonia and remained febrile, although two received antimicrobial therapy for the positive cultures.

The *Bacillus* spp. from all five patients appeared as gram-negative, nonspore forming rods in smears from the original blood culture bottles (BACTEC NR6A; Becton Dickinson Diagnostic Instrument Systems, Towson, Md.). Subculturing on 5% sheep blood agar (BBL Microbiology Systems, Cockeysville, Md.) revealed translucent colonies with oval, swollen spores after 48 h of incubation at 37°C in 5% CO_2.

Identification of strains. All five *Bacillus* isolates were motile, exhibited catalase activity, lacked hemolysins, lacked lecithinase activity, and were inhibited by penicillin (zone diameters of greater than 36 mm). Isolates from patients 5, 6, 7, and 8 had mucoid colony morphology. Isolate 9 was dry and flat. With the identification schemes of Hollis and Weaver (11), isolates from patients 5, 6, and 9 were identified to the species level as *Bacillus circulans*. Spore size and location, starch hydrolysis, growth at 25°C, indole and H_2S production, gas from glucose, and the Voges-Proskauer reaction were key reactions for identification.

Environmental investigation. Potential sources of culture contamination in OPBDS were investigated. No organisms were isolated from swab cultures of the septa of uninoculated BACTEC bottles or heparin-containing tubes used for mycobacterial blood cultures (VACUTAINER tubes; Becton Dickinson Vacutainer Systems, Rutherford, N.J.).

Ten nonsterile gloves from the OPBDS were cultured by placing them on the hand and touching the tips of the fingers to tryptic soy agar with 5% sheep blood. These fingertip cultures showed 20 to 70 colonies of *Bacillus* spp., including *B. circulans*, per fingertip (Fig. 1). Similar cultures of 10 gloves per lot number from two other distributors showed no colonies after 48 h of incubation.

Quantitative glove cultures were performed. With a sterile technique, gloves were removed from an unopened box and cut into pieces, which were placed in 200 ml of brain heart infusion (BBL) and dispersed by sonication for 2 min. Aliquots totaling 1 ml of the solution were plated onto 5% sheep blood agar. Ten milliliters of each solution was also filtered through 0.45-μm-pore filters, and the filters were incubated on sheep blood agar. An additional 10 ml of the

TABLE 1. Isolation of *Bacillus* spp. from blood cultures from 1 July 1985 to 1 July 1987

Patient	Date that specimen was taken (mo/day/yr)	Ward that specimen was collected on	Bottle type	No. with *Bacillus* isolates/no. cultured	Sex[a]	Diagnosis	Identification
1	10/12/85	A	6B	1/2	F	AIDS	*Bacillus* spp.
2	3/15/86	B	6B	1/1	F	Leukemia	*Bacillus* spp.
3	5/30/86	OPBDS	6B	1/2	F	AIDS	*Bacillus* spp.
4	6/3/86	OPBDS	NR6A	1/2[b]	F	Pyloric stenosis	*Bacillus* spp.
5	11/4/86	OPBDS	NR6A	1/2	M	AIDS	*B. circulans*
6	12/2/86	OPBDS	NR6A	1/2	M	AIDS	*B. circulans*
7	2/2/87	OPBDS	NR6A	1/2	M	AIDS	*Bacillus* spp.[c]
8	2/24/87	OPBDS	NR6A	1/2	M	AIDS	*Bacillus* spp.[c]
9	3/13/87	OPBDS	NR6A	1/2	M	AIDS	*B. circulans*
10	6/4/87	ER[d]	NR6A	1/4	M	AIDS	*Bacillus* spp.

[a] F, Female; M, male.
[b] *Staphylococcus epidermidis* was also present in the culture.
[c] Colonies were morphologically identical to *B. circulans*.
[d] ER, Emergency room.

sample was heated to 80°C for 20 min, and cultures were repeated as described above.

With the quantitative culture technique, the gloves from the lot used in the OPBDS were shown to contain 110,000 CFU per glove; after heating to 80°C, the yield of organisms was 170,000 CFU per glove, indicating that the contamination was due to the spore form and not to the vegetative form. No *Bacillus* spp. were recovered from a different lot of nonsterile gloves from the same distributor or from nonsterile gloves from two other distributors.

The isolation of *Bacillus* spp. from AIDS patients was of clinical concern, especially since the preliminary laboratory data suggested that the individuals were potentially infected with gram-negative organisms. Nonanthrax *Bacillus* spp. have been implicated in human disease, particularly in immunocompromised hosts (5, 7, 8, 12, 18). The first two patients in the 5-month cluster became afebrile in association with antimicrobial therapy, but the last three patients did not.

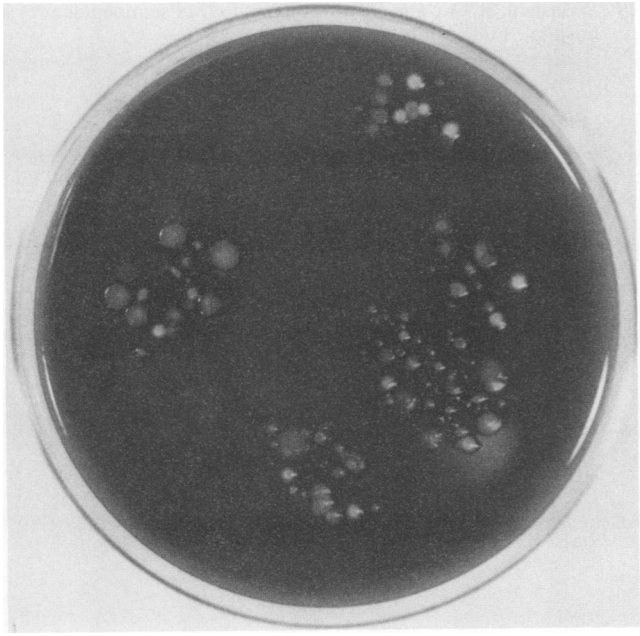

FIG. 1. Sheep blood agar plate after inoculation with the fingertips of nonsterile gloves from a contaminated lot. Plates were incubated for 48 h at 37°C in 5% CO_2. Each colony type was examined microscopically and stained as gram-positive sporeforming bacilli.

A pseudoepidemic was suspected by the Infectious Disease Service of the University of California Medical Center after cases 7 and 8 were reported on the basis that the clinical outcome did not correlate with the microbiological findings. Other evidence suggested a pseudoepidemic: the *Bacillus* isolates lacked the virulence factors (lecithinase, hemolysins, and penicillinase) associated with true bacteremia (7, 18), only one documented case of *B. circulans* associated with disease has been reported (2), and it is statistically unlikely that true bacteremia is present unless multiple cultures are positive (5).

At the time that cultures were done for the patients, the use of gloves was recommended whenever there was a possibility of exposure to body fluids. The biased recovery of these contaminants from AIDS patients most likely reflected the practice at the time of wearing gloves for phlebotomy only for those patients suspected of being infected with human immunodeficiency virus type 1. Isolation of *B. circulans* from the gloves used in the OPBDS during phlebotomy and from at least three of the five positive blood cultures implicated contaminated gloves as the cause of the positive blood cultures.

Cornstarch used on gloves can contain gram-positive sporeforming organisms; the acceptable level for many manufacturers of cornstarch used for nonsterile gloves is less than 600 total bacteria per g (Lowell E. Coker, personal communication). Since the amount of starch per glove is far less than a gram, the number of organisms on the contaminated gloves implicated in this investigation clearly exceeded the acceptable bacterial count.

The BACTEC 460 blood culture system has been implicated in several pseudoepidemics involving sporeforming species (13). The bottles which yielded *Bacillus* spp. were processed at intervals several weeks apart, and no bottle with *Bacillus* spp. had been tested on the BACTEC concurrently with another bottle with *Bacillus* spp. Consequently, the BACTEC could not be implicated in the pseudoepidemic.

Unnecessary hospitalizations and antimicrobial usage resulted from the pseudoepidemic partly because the *Bacillus* spp. originally stained gram negative in the blood culture broth, and until the colonies were isolated, they were reported as gram-negative rods. This staining phenomenon is not surprising, since *B. circulans* typically stains gram

negative (17). Three of the five patients in this pseudoepidemic were initially treated for gram-negative sepsis. Three patients were later treated for *Bacillus* sepsis. Two patients were admitted to the hospital solely because of the report of a positive blood culture. It was only after the demonstration of the same *Bacillus* spp. on the gloves and in the bottles that the physicians definitively dismissed *Bacillus* spp. as opportunistic pathogens in AIDS patients.

With the extensive use of glove wearing as a safety measure for laboratory personnel, it is critical that microbiologists be aware that nonsterile gloves can contain *Bacillus* spores which can contaminate specimens collected for microbiological culturing and which can frequently appear as gram-negative organisms in initial cultures.

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