JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1997, p. 2040–2042 Vol. 35, No. 8
0095-1137/97/$04.00+0

Pseudo-Outbreak of Septicemia Due to Rapidly Growing Mycobacteria Associated with Extrinsic Contamination of Culture Supplement

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Received 29 April 1997/Accepted 16 May 1997

Between April and December 1994, 23 blood cultures from human immunodeficiency virus-infected patients grew rapidly growing mycobacteria suspected to be *Mycobacterium chelonae* at a hospital in New Jersey. The isolates were later identified as *M. abscessus*. Several bacterial species, including *M. abscessus*, were cultured from an opened multidose supplement vial (BBL Septi-Chek AFB Supplement) that had been used for mycobacterial blood cultures. The *M. abscessus* isolates from case patients and the supplement vial had identical multilocus enzyme electrophoresis and antimicrobial susceptibility patterns. Finding a contaminated vial of supplement, together with the lack of a distinct syndrome in case patients, was consistent with a pseudo-outbreak.

Mycobacterium chelonae formerly contained two subspecies, *M. chelonae* subsp. *chelonae* and *M. chelonae* subsp. *abscessus*. The subspecies have now been determined to warrant differentiation at the species level. The two species are closely related and are found free living in water and soil. Approximately 90% of the clinical disease caused by rapidly growing mycobacteria is due to *M. fortuitum*, *M. chelonae*, or *M. abscessus* (18). Human infection is usually a posttraumatic event following penetrating wounds or surgery. The presence of mycobacteria in water supplies may complicate laboratory diagnostic tests because the bacilli can contaminate reagents and vessels, leading to false-positive results when patient specimens are tested for mycobacteria. For example, pseudo-outbreaks of disease due to *M. chelonae* have been traced to contamination of bronchoscopes used to obtain bronchial washings (7).

From April 1994 to December 1994, 23 blood cultures positive for *M. chelonae* were identified by microbiology laboratory personnel at a single New Jersey hospital (hospital A). Isolation of *M. abscessus* from a large number of blood specimens processed at a single hospital laboratory was unusual, and the finding was potentially meaningful because the patients were human immunodeficiency virus (HIV) infected, a condition often complicated by mycobacterial infections. The presumed outbreak of septicemia due to *M. abscessus* could have indicated a source of infection that was not recognized previously.

The objectives of our investigation were to define any associated morbidity, to determine the rate of nontuberculous mycobacteremias in the HIV-infected population served by the hospital, and to determine risk factors for infection. A case-control study did not reveal evidence of disease among those case patients with positive cultures, and the investigation focused on determining if the isolates were contaminants.

MATERIALS AND METHODS

Epidemiologic investigation. (i) Case definition and ascertainment. A case was defined as a blood culture positive for *M. abscessus* between October 1993 and December 1994 in an HIV-infected patient from hospital A. To identify case patients and determine the background rate of *M. abscessus*-positive blood cultures among hospital patients, we reviewed the hospital microbiology laboratory records during the study period. Information collected on each specimen included the patient's name, the specimen number, hospital service, date and time of collection, and the results of mycobacterial culture.

(ii) Case-control study. To define morbidity and identify risk factors for an *M. abscessus*-positive blood culture, we conducted a case-control study. We compared each case patient with two controls. For each case patient, the next two HIV-positive patients who had sterile blood cultures on the same day were selected as controls.

(iii) Statistical analysis. Data were collected on standardized forms, entered, and analyzed by using Epi Info version 6.01 (Centers for Disease Control and Prevention, Atlanta, Ga.). Categorical variables were compared by using the chi-square test or Fisher's exact test. Continuous variables were evaluated by using the Kruskal-Wallis test. Odds ratios, 95% confidence intervals, and *P* values were calculated.

Environmental investigation. (i) Procedure review. To detect potential mechanisms of contamination, specimen collection procedures in the hospital and in the outpatient facility were observed. All laboratory personnel were interviewed and observed processing blood cultures for mycobacteria. In addition, infection control staff and clinicians were interviewed regarding specimen collection techniques.

(ii) Environmental culture survey. To assess the possibility of an environmental source of *M. abscessus*, we cultured all of the reagents used in the blood culture process, commercially available sterile water used for reconstituting reagents, tap water, and selected environmental sites, including surfaces of the hood faucets and incubators. Surfaces were swabbed with individual, sterile, cotton-tipped applicators moistened with sterile water. Tap water specimens were collected from individual taps into sterile plastic containers. All samples were kept cool and processed within 2 days of collection.

Swabs and water samples were inoculated onto Middlebrook 7H11 mycobacterial medium and observed for growth. The 23 isolates were originally misidentified as *M. chelonae* by the hospital A referral laboratory. Of the 23 isolates identified during the study period, only 9 were available for further analysis. The remaining isolates had been discarded.

(iii) Characterization of isolates. The nine available isolates were further characterized by high-pressure liquid chromatography, drug susceptibility tests, and typing by multilocus enzyme electrophoresis (4, 20).

For each isolate, MICs of 13 antimicrobial agents were determined by broth microdilution; the agents tested were imipenem, ciprofloxacin, augmentin, cefoxitin, amikacin, tobramycin, kanamycin, clarithromycin, minocycline, erythromycin, sulfamethoxazole, capreomycin, and rifampin (14).

For multilocus enzyme electrophoresis, metabolic enzymes were separated on 11% starch gels and stained as previously described (20). The enzymes analyzed were isocitrate dehydrogenase, phosphogluconate dehydrogenase, glucose 6-phosphate dehydrogenase, benzyl alcohol dehydrogenase, diaphorase, indophenol oxidase, glutamate oxaloacetic transaminase, adenylate kinase, phosphoglucose mutase, esterase, leucine aminopeptidase, and phosphoglucose isomerase. Each enzyme variant was assigned an allele number in ascending order based on increased migration toward the anode. Strains were assigned to an electrophoretic type based on the combination of allele numbers.

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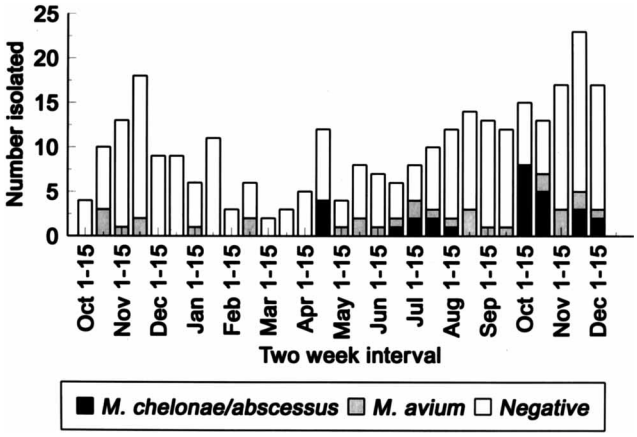

FIG. 1. Results of blood cultures for nontuberculous mycobacteria between 15 October 1993 and 15 December 1994 in hospital A.

RESULTS

Descriptive epidemiology. During the study period, 291 blood specimens were submitted to the hospital A microbiology laboratory for mycobacterial culture (Fig. 1); 54 (19%) were positive for mycobacteria. Positive cultures included the *M. avium* complex ($n = 31$) or *M. chelonae* ($n = 23$). The frequency of isolation of the *M. avium* complex over the study period was constant. From April to December 1994, *M. chelonae* was isolated from 23 (8%) of 291 blood cultures. However, *M. chelonae* had not been isolated from blood before April 1994, when a new mycobacterial blood culture technique was introduced in the laboratory. In April, July, and October 1994, clusters of blood cultures positive for *M. chelonae* occurred (Fig. 1). In October 1994, *M. chelonae* was isolated from 13 (45%) of the 29 blood cultures processed.

Environmental investigation. (i) Procedure review. Before April 1994, all blood cultures for mycobacteria were referred to a commercial reference laboratory. In April 1994, the hospital instituted in-house testing with the combination of the Isostat-10 lysis centrifugation Vacutainer tube (Wampole Laboratories, Cranberry, N.J.) and the Septi-Chek AFB biphasic culturing system (Becton Dickinson, Cockeysville, Md.). The Septi-Chek AFB culture system consists of (i) a bottle containing liquid medium (30.0 ml of Middlebrook 7H9 broth), (ii) a paddle with agar medium enclosed in a capped plastic tube, and (iii) a supplement bottle containing lyophilized glucose, glycerin, oleic acid, pyridoxal, catalase, albumin, polyoxyethylene 40 stearate, azlocillin, nalidixic acid, trimethoprim, polymyxin B, and amphotericin B.

The Septi-Chek system was processed by first resuspending the lyophilized supplement in 9 ml of sterile water obtained from a 1-liter bottle of sterile, distilled water stored in the common refrigerator in the central microbiology laboratory. This water served as the common water source used for resuspending media and reagents for the microbiology laboratory.

Interviews of laboratory personnel revealed that their inoculation techniques varied, and observation confirmed inconsistencies in performance. Resuspension of the supplement was performed on the open bench by one of three technicians. The rubber caps of the vials were not always disinfected before entry, and a pipette that touched the surface of the hood was used by one technician despite risk of contamination.

(ii) Characterization of isolates. Nine mycobacterial isolates were available from the blood cultures of 23 case patients. Seven of the nine blood culture isolates from case patients were identified as *M. abscessus*; the two others were identified as *M. mucogenicum*. Several bacteria, including *M. abscessus*, *M. fortuitum*, and other, nonmycobacterial species, were cultured from an in-use supplement vial.

The *M. abscessus* strain recovered from the supplement vial was identical by isoenzyme assay and antimicrobial susceptibility patterns to the seven case patient isolates (Table 1). In addition, homology between the supplement and the case patient isolates by isoenzyme analysis was 100% for the 13 enzymes screened.

No bacteria were recovered from three previously unopened supplement vials. No other environmental cultures were positive for mycobacteria.

DISCUSSION

M. chelonae was first recovered in 1930 by Friedmann from a sea turtle (*Chelonae corticata*) (8). The first human isolate was reported in 1953 and was associated with a septic joint (12). Since the early 1970s, there have been several reports of clinical disease associated with *M. chelonae*. The most common sites of infection due to these rapidly growing mycobacteria are the skin (13, 15, 17) and lungs (9, 16). Nosocomial infections have been associated with ear examination (11), peritoneal dialysis (2, 3), needleless injection, and bronchoscopy (19).

The recent increase in *M. tuberculosis* infection and other mycobacterial diseases associated with the HIV epidemic has renewed interest in the development of rapid and sensitive

TABLE 1. Identification and susceptibility patterns of mycobacteria isolated from case patients and a Septi-Chek Supplement vial

Strain	Source	Identification	MIC (μg/ml) of antimicrobial agent[a]												
			IMI	CIP	AUG	CX	AK	TO	KAN	CLA	MN	E	SMX	CAP	RIF
94-2633	Patient	*M. abscessus*	>32	16	128	16	>32	>32	32	4	>32	>32	512	>128	>16
94-2634	Patient	*M. abscessus*	>32	16	128	16	>32	>32	16	2	32	32	256	>128	16
94-2690	Patient	*M. abscessus*	>32	16	128	16	16	16	8	2	>32	32	512	>128	>16
94-2712	Patient	*M. abscessus*	>32	16	128	8	32	32	16	1	>32	32	512	>128	>16
94-2713	Patient	*M. abscessus*	>32	>16	128	32	32	32	16	2	>32	>32	512	>128	>16
94-2714	Patient	*M. abscessus*	>32	16	64	8	32	32	16	2	>32	>32	512	>128	>16
94-2885	Patient	*M. abscessus*	>32	16	128	16	32	32	16	1	>32	32	512	>128	>16
C	Supplement	*M. abscessus*	>32	>16	>128	64	16	16	8	1	>32	>32	512	>128	>16
95-0094	Patient	*M. mucogenicum*	32	0.25	8	16	4	4	4	0.25	0.25	16	16	>128	2
95-0097	Patient	*M. mucogenicum*	>32	1	16	32	4	8	4	0.5	0.25	32	16	128	8
B	Supplement	*M. fortuitum*	2	1	128	64	8	32	>32	32	1	>32	32	64	16

[a] Abbreviations: IMI, imipenem; CIP, ciprofloxacin; AUG, augmentin; CX, cefoxitin; AK, amikacin; TO, tobramycin; KAN, kanamycin; CLA, clarithromycin; MN, minocycline; E, erythromycin; SMX, sulfamethoxazole; CAP, capreomycin; RIF, rifampin.

techniques for culture and identification of mycobacteria. Solid-medium systems require 3 to 6 weeks of incubation and have been shown to have higher minimal detection levels (1, 10). The BACTEC 460 radiometric system, which uses liquid medium, has facilitated improved recovery and decreased identification time. However, the expense of this system may be prohibitive for some clinical laboratories, particularly those processing small numbers of cultures (1, 10). The Septi-Chek AFB system was developed as a dual-phase system that does not require either the radiometric detection system or the disposal of radioactive waste that is associated with the BACTEC system (5, 10). In addition, the Isostat tube has been used for the isolation of rapidly growing mycobacteria from blood (6).

The use of a lysis centrifugation tube (Isostat tube) requires three separate manipulations that could lead to the introduction of contaminating microorganisms. These manipulations include application of the specialized cap, entry of a pipette used to aspirate the supernatant, and entry of a separate pipette to aspirate the sediment. The Septi-Chek AFB system requires four separate manipulations that could lead to the introduction of contaminating organisms. When these two systems are used together, the original sample is manipulated seven times before incubation. Furthermore, in this laboratory, the 1-liter sterile water bottle was reused over periods of 1 to 2 weeks.

In our investigation, the lack of a distinct clinical syndrome attributable to suspected *M. abscessus* bacteremia, the high rates of isolation of rapidly growing mycobacteria from blood, the clustering of positive *M. abscessus* blood cultures, and the finding of contaminated reconstituted supplement were all consistent with laboratory contamination.

Our observations of the manipulation of mycobacterial cultures in the hospital's microbiology laboratory, the culturing of two mycobacterial strains from an in-use supplement vial, and the finding of sterile, unopened supplement vials suggest that the supplement was extrinsically contaminated. How the in-use supplement bottle became contaminated is not known. Our observations of laboratory procedures suggest several potential points of entry for contaminating mycobacteria, including manual removal of the injection cap of the bottle, repeated entry of an injection cap bottle, multiple use of a common sterile water source, and lapses in sterile technique during specimen processing.

In the microbiology laboratory, multiuse bottles should be kept to a minimum. If reuse is unavoidable, such bottles should be handled with a strict aseptic technique and periodically screened for microbiologic contamination, including nontuberculous mycobacteria. Active surveillance should be maintained for clusters of unusual organisms or higher rates of recovery of common species. Clusters of an unusual organism should lead to an immediate investigation.

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