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Isolation of *Legionella pneumophila* Serogroup 1 from Blood with Nonsupplemented Blood Culture Bottles

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Legionella pneumophila serogroup 1 was isolated from antemortem aerobic blood culture bottles not supplemented with L-cysteine or ferric pyrophosphate and from the postmortem lung tissue of a 72-year-old man. We recommend that aerobic blood culture bottles (Johnston Laboratories, Cockeysville, Md.) of BACTEC be subcultured to an agar that supports the growth of *L. pneumophila* when growth indexes range from 30 to 60 but fail to increase further.

Legionella pneumophila has been proven responsible for a substantial number of pneumonia cases (2). Frequently, systemic manifestations, e.g., fever, headache, and diarrhea, accompany Legionnaires disease, (6). Nevertheless, *L. pneumophila* has been isolated from blood cultures on only two occasions before this report (1, 7). The scarcity of isolations may, in part, be due to the failure of laboratories to consider the possibility of members of the family *Legionellaceae* when blood culture bottles not supplemented with L-cysteine and ferric pyrophosphate are used. Hopefully, our recovery of *L. pneumophila* from routine blood culture bottles (no L-cysteine or ferric pyrophosphate added), even after 7 days of incubation, will encourage microbiologists who use the BACTEC blood culture system (Johnston Laboratories, Cockeysville, Md.) to subculture aerobic vials that give low-level growth index (GI) readings (30 to 60) to an agar capable of growing *L. pneumophila*.

CASE REPORT

A 72-year-old man with a history of interstitial pulmonary fibrosis, atherosclerotic heart disease with myocardial infarction and diabetes mellitus was admitted to the Biscayne Medical Center, Miami, Fla., on 28 May 1982. One week earlier, the patient had been discharged from another hospital where a diagnosis of mixed collagen vascular disease had been made. On the basis of that diagnosis, the patient was placed on prednisone (60 mg per day in divided doses).

When the patient was admitted to the Biscayne Medical Center, he was in severe respiratory distress, with a pulse rate of 130, a respiratory rate of 36, and temperature of 100.2°F (37.8°C). A chest X ray revealed a right lower lobe infiltrate suggestive of pneumonia and pulmonary edema. Arterial blood gas at the time of admission revealed a pH of 7.43, a partial CO_2 pressure of 34, and a partial O_2 pressure of 74. The patient was not on oxygen therapy.

The clinical diagnosis at the time of admission was pneumonia complicating congestive heart failure; the patient was placed in the intensive care unit. He was given antibiotics, initially tobramycin, 80 mg intravenously every 12 h, and ticarcillin, 3 g intravenously every 4 h but he was later given cefamandole, 2 g intravenously every 6 h. Before the initiation of antibiotic therapy, three blood cultures were drawn from separate venipuncture sites.

Despite measures to correct the congestive heart failure and despite antibiotic therapy for the pneumonia, the patient continued to run temperatures ranging between 101°F (38.3°C) and 102°F (36.9°C), with continued pulmonary consolidation of the right lower lobe. Furthermore, the patient became progressively hypotensive despite dopamine infusions to maintain blood pressure. On day 3 after admission, endotracheal aspirations revealed a reddish-brown material that tested positive for blood. The general condition of the patient continued to deteriorate, and he experienced several episodes of ventricular arrhythmia, necessitating a lidocaine drip. Subsequently, the patient developed sinus bradycardia, with a pulse rate of 52 prompting cardiopulmonary resuscitation. Despite resuscitative measures, the patient expired on hospital day 5.

Permission for an autopsy confined to the heart and lungs was granted. The postmortem examination revealed bilateral pulmonary edema and right lower lobe consolidation. Portions of the right lower lobe consolidation were submitted for bacterial, mycobacterial and fungal culturing and Formalin-fixed material was submitted for routine histological sectioning.

MATERIALS AND METHODS

Culture media. We used commercially prepared aerobic and anaerobic BACTEC test vials (6B and 7C, respectively), subsequently referred to as blood culture bottles. The aerobic 6B bottle contained a 10%

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TABLE 1. GI readings of BACTEC blood culture bottles containing *L. pneumophilia* over the 7-day incubation period

Expt	Bottle	GI on indicated day of incubation						
		1	2	3	4	5	6	7
1	Aerobic	21[a]	36	44	40	38	35	30
	Anaerobic		6	7	7	7	5	4
2	Aerobic	23	35	44	42	40	35	32
	Anaerobic		8	9	8	5	5	5
3	Aerobic	24	38	49	46	43	36	32
	Anaerobic		7	6	7	7	7	4

[a] A GI of ≥30 with aerobic bottles constitutes a positive reading, and a GI of ≥20 with anaerobic bottles constitutes a positive reading.

(wt/vol) CO_2 atmosphere but no L-cysteine HCl. The anaerobic 7C bottle contained a 10% CO_2–90% N_2 atmosphere and L-cysteine HCl. Isolation agar was buffered charcoal-yeast extract (BCYE) agar (Remel, Lenexa, Kans.).

Blood culture methodology. On the day of admission, 3 10-ml volumes of blood, each 20 min apart, were drawn from the patient by an aseptic technique. Each 10-ml volume was divided into two 5-ml amounts, one amount inoculated into a 6B bottle and the other into a 7C bottle. The three aerobic 6B bottles were incubated at 35°C with agitation on a BACTEC shaker on day 1 of incubation and without agitation on days 2, 3, 4, 5, 6, and 7 of incubation. Each aerobic 6B bottle was sampled daily with a BACTEC 460 instrument. The three anaerobic 7C bottles were incubated at 35°C without agitation on all 7 days of incubation and sampled on days 2, 3, 4, 5, 6, and 7 of incubation.

The positive GIs used with the BACTEC machine were ≥30 for the aerobic 6B bottles and ≥20 for the anaerobic 7C bottles.

All cultures which were radiometrically positive on days 2 and 3 of incubation were Gram stained and subcultured to chocolate agar plates, which were then incubated at 35°C for 48 h in 5% CO_2, and to blood agar plates, which were then incubated anaerobically (GasPak jar, BBL Microbiology Systems, Cockeysville, Md.) at 35°C for 48 h.

On day 7 of incubation, after the isolation of *L. pneumophila* from the patient's lung tissue was confirmed (see results below), each of the aerobic and anaerobic bottle cultures was Gram stained, subcultured to BCYE agar, and incubated at 35°C in a candle extinction jar.

Lung tissue methodology. On hospital day 5 the patient expired. Lung tissue was obtained from autopsy, minced with a sterile scalpel, and inoculated to the following agars: blood, chocolate, and MacConkey (all incubated in 5% CO_2); BCYE (candle extinction jar); and Schaedler, Schaedler kanamycin-vancomycin, and colistin-nalidixic acid (all incubated anaerobically). A gram stain of the minced tissue was done.

Sputum cultures. Sputa submitted for culturing on hospital days 1 and 2 were inoculated to blood, chocolate, MacConkey, and colistin-nalidixic acid agars (all incubated in 5% CO_2).

Biochemical identification tests. Oxidase, catalase and gelatinase tests were performed as recommended by the Centers for Disease Control, Atlanta, Ga. (13).

RESULTS

Isolation from lung tissue. The Gram stain of the lung tissue did not show bacteria. However, approximately 50 colonies were growing on the BCYE agar after 48 h of incubation. No growth was visible on any of the other aerobically or anaerobically incubated agars, even after 72 h of incubation. The BCYE agar colonies consisted of gram-negative bacilli which ranged in length from 3 to 10 μm and somewhat resembled fusobacteria. Subcultures from the BCYE agar to blood and chocolate agars failed to grow, but visible growth was seen in confluent areas on a subculture to a second BCYE agar after overnight incubation. The colonies were positive for the oxidase, catalase, and gelatinase tests. The Special Bacteriology Section of the Centers for Disease Control confirmed the lung isolate and both blood isolates as *L. pneumophila*. The Immunofluorescence Section of the Centers for Disease Control subsequently identified all three isolates as *L. pneumophila* serogroup 1.

Isolation from blood cultures. Table 1 shows the BACTEC 460 GI determinations from days 1 through 7 of incubation for each of the aerobic and anaerobic bottles. On day 2, each of the three aerobic bottles had GI values that suggested positive cultures. Gram stains of the three aerobic bottle cultures and the three anaerobic bottle cultures failed to demonstrate bacteria. Subcultures of the broth from the six bottles to blood and chocolate agars failed to grow any bacteria. On day 3, as the GI values of the aerobic bottle cultures continued to increase, Gram stains and subcultures were done again, with the same negative results. Upon recovery of the lung tissue isolate, corresponding to day 7 of the blood culture incubation, all cultures from the six bottles were subcultured to BCYE agar, and *L. pneumophila* serogroup 1 was recovered from two of the three aerobic bottle cultures (ca. 15 colonies on each agar) but from none of the anaerobic bottle cultures. Again, Gram stains of

the cultures from the six bottles did not show bacteria.

Routine sputum cultures. Both sputum cultures grew *Staphylococcus aureus* and *Serratia marcescens*.

DISCUSSION

The isolation of *L. pneumophila* from the lung tissue of persons who died during the 1976 outbreak in Philadelphia was accomplished with Mueller-Hinton agar supplemented with IsoVitaleX (BBL Microbiology Systems) and hemoglobin (8). It was subsequently determined that L-cysteine could replace the IsoVitaleX component (4). Most studies since have stressed the L-cysteine requirement of *L. pneumophila* as well as the stimulatory effect upon its growth by ferric pyrophosphate (5, 9–12). The concept of L-cysteine and ferric pyrophosphate supplementation was further strengthened by the two previous isolations from blood of *L. pneumophila* with blood culture bottles specifically supplemented with these two components (1, 7). Presently, the use of supplemented blood culture bottles is recommended when attempting to recover members of the family *Legionellaceae* from blood (3). Faced with this information, microbiologists must logically conclude that special blood culture broth must be employed when attempting to recover *L. pneumophila* from blood and that the use of routine blood culture bottles for this purpose is futile. Our experience demonstrates that *L. pneumophila* can be recovered from nonsupplemented blood culture bottles. It is entirely possible that a patient's blood may furnish sufficient quantities of iron and L-cysteine for the survival of *L. pneumophila*.

Had we been aware of the ability of *L. pneumophila* to survive in and be recovered from routine blood culture bottles even after 7 days of incubation, and confronted with radiometrically positive blood cultures for a 72-year-old pneumonia patient, we would have subcultured the blood cultures to BCYE agar in addition to blood and chocolate agars after overnight incubation. Thus, on hospital day 3 or 4 growth of gram-negative bacilli would have been discerned on BCYE agar but not on blood or chocolate agar, a situation that strongly suggests the presence of the Legionnaires disease bacillus. Bearing in mind that the patient expired on hospital day 5, the laboratory diagnosis of Legionnaires disease on day 3 or 4 could have resulted in more timely and appropriate therapy, i.e., erythromycin.

In view of our experience, we recommend subculturing of BACTEC bottles to agar that supports the growth of *L. pneumophila* when GI readings range from 30 to 60 and subsequently fail to increase or perhaps begin to fall. We further recommend that such subculturing be limited to cultures from aerobic bottles since we recovered *L. pneumophila* from cultures from the aerobic bottles (L-cysteine free) but not from the anaerobic bottles (L-cysteine supplemented). This contention is supported by Saito et al. (11), who found that "moderate aeration resulted in a faster growth rate and in approximately a 1 $\log_{10}$ higher final cell concentration (of *L. pneumophila*) as compared to a static broth culture."

In addition to helping to confirm a diagnosis of Legionnaires disease, subculturing of BACTEC blood culture bottles that give low-level GI readings to agar that supports the growth of *L. pneumophila* may produce an unsuspected diagnosis with life-saving results.

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