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Isolation of *Mycobacterium chelonei* with the Lysis-Centrifugation Blood Culture Technique

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Mycobacterium chelonei was isolated from a patient by the lysis-centrifugation and the conventional two-bottle blood culture methods. The lysis-centrifugation method was significantly more sensitive and rapid than the conventional method in detecting and isolating this organism; quantitations done by this method were useful for monitoring response to therapy.

The lysis-centrifugation (L-C) blood culture technique described by Dorn and Smith (2) has been shown to be an efficient and rapid method for the detection and isolation of bacteria and fungi from the blood of patients (1). With this method, blood is inoculated into a double-stoppered, evacuated tube (ISOLATOR; E. I. du Pont de Nemours & Co., Inc.) containing sodium polyanethol sulfonate, EDTA, and saponin, which lyses the erythrocytes. After centrifugation, most of the fluid is removed and discarded. The residual fluid and cellular debris are blended on a Vortex mixer and then withdrawn and inoculated onto appropriate media. During a recent evaluation of this device, a case was encountered in which the detection and isolation of *Mycobacterium chelonei* from the blood of a renal transplant patient was significantly facilitated.

A 33-year-old woman was followed since 1978, when bilateral nephrectomy was performed for Goodpasture's syndrome. She underwent hemodialysis until she received a cadaveric renal transplant in July 1981. Immunosuppression was performed 1 month before transplantation with corticosteroids and T-lymphocyte depletion. She became febrile after surgery, and *Pseudomonas acidovorans* was isolated from her blood. In spite of therapy, she developed signs and symptoms of peritonitis. On day 6 after transplantation, an exploratory laparotomy revealed a ureterovesicle leak at the anastomotic site, and this was repaired. The patient improved briefly, but on day 10 after transplantation, she again became febrile, and blood cultures were taken by a conventional two-bottle method. In the conventional method, paired sets of blood culture bottles containing 100 ml of Columbia broth or 100 ml of tryptic soy broth, each of which was inoculated with 10 ml of blood, were used. The bottles were incubated at 37°C for 7 days and examined macroscopically daily and microscopically with the acridine orange technique (6) within the first 24 h. Blind subcultures were performed after 24 and 48 h and 7 days; subcultures were held for 48 h each time.

From these conventional blood cultures, a slow-growing organism was isolated which stained gram positive in a beaded pattern resembling gram-positive cocci in chains. The organism was subsequently shown to be an acid-fast bacillus and was identified as *M. chelonei* subsp. *abscessus* on the basis of the following characteristics: no pigment, growth in less than 7 days, growth on MacConkey agar which did not contain crystal violet, growth in the presence of 5% NaCl, positive arylsulfatase in 3 days, positive catalase, negative nitrate reductase, and negative iron uptake. Identification of this organism was confirmed by the Texas State Department of Health Laboratories. Antimicrobial susceptibility was determined by a disk diffusion method (8). The organism was found to be susceptible to amikacin and kanamycin and resistant to gentamicin and tobramycin.

The patient became afebrile on amikacin therapy, and she was discharged 1 month after renal transplantation. A month later, she was readmitted for evaluation of the progression of peripheral subcutaneous nodules which had appeared initially during her previous hospitalization. Culture of a nodule biopsy specimen yielded *M. chelonei*. Although she was afebrile, blood cultures by the conventional method and the L-C technique were obtained shortly after readmission. The media initially used for the L-C technique consisted of Columbia blood agar and chocolate blood agar incubated in CO_2 at 35°C, prereduced blood agar supplemented with hemin and vitamin K and incubated anaerobically at 35°C, and Sabouraud dextrose agar incubated

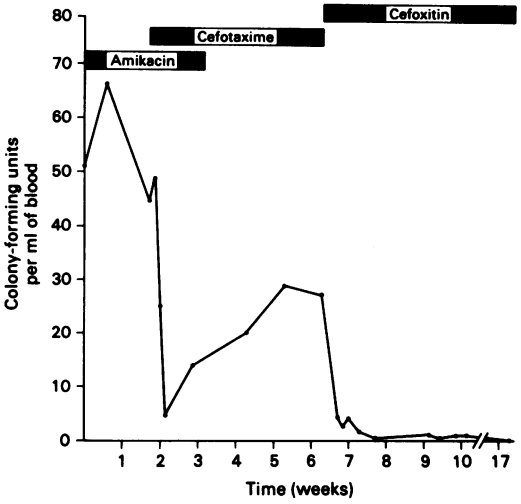

FIG. 1. Quantitation by the L-C technique of *M. chelonei* in blood specimens collected during antimicrobial therapy.

aerobically at 30°C. All cultures were incubated for 8 days.

M. chelonei was detected and isolated in 44 h by the L-C technique and in 7 days by the conventional method. For the L-C blood cultures, the organism grew best on Columbia blood agar and poorest on Sabouraud dextrose agar and did not grow anaerobically. (As a result of this observation, Columbia blood agar was used exclusively for subsequent L-C blood cultures from this patient.) In addition, quantitation done with the L-C method revealed nearly 50 colony-forming units (CFU) per ml.

Over the next several weeks, a total of 45 blood cultures were done by the L-C method, and 17 cultures (34 bottles) were done simultaneously by the conventional method to monitor the response to therapy. All blood cultures done by the L-C method yielded *M. chelonei* in 60 to 65 h, whereas only 10 of 34 (29.4%) conventional broth cultures were positive; when they were positive, they were detected after 7 days of incubation. Conventional cultures were positive only when the concomitant L-C cultures demonstrated greater than 25 CFU/ml. Thus, the L-C technique provided faster and more sensitive detection and isolation of the mycobacterium.

The ability of the L-C technique to quantitate the level of mycobacteremia in this patient was useful in monitoring the response to therapy (Fig. 1). *M. chelonei* counts initially ranged from 50 to 70 CFU/ml. The counts dropped to about 45 CFU/ml after nearly 2 weeks of amikacin therapy and to 5 CFU/ml after several days of combined amikacin and cefotaxime therapy. However, the *M. chelonei* counts then increased again in spite of continued amikacin plus cefotaxime therapy or cefotaxime therapy alone, and the counts returned to 25 to 30 CFU/ml after 5 to 6 weeks of treatment. By this time, results of minimum inhibitory concentration testing of an isolate from a subcutaneous nodule had become available. The minimum inhibitory concentrations were determined by the standard tube dilution method with the following results (in micrograms per milliliter): amikacin, 8.0; kanamycin, 8.0; tobramycin, 8.0; cefoxitin, 16.0; and erythromycin, 4.0. The third-generation cephalosporins cefotaxime, moxalactam, ceftazidine, and cefoperazone were ineffective at the highest concentrations tested (R. J. Wallace, Jr., Baylor College of Medicine, personal communication). Since the patient was not improving, treatment was changed to cefoxitin. Within 1 week, the quantitative counts had decreased to 0.2 to 2.0 CFU/ml, and they remained at this level for several weeks. Blood cultures became totally negative for *M. chelonei* after removal of the transplanted kidney and one of the arteriovenous fistulas used for hemodialysis.

Mycobacteria of the *M. fortuitum-chelonei* complex are almost ubiquitous in the environment. They are being increasingly reported as human pathogens which cause such problems as soft tissue abscesses, subcutaneous nodules, wound infections, and rarely, pulmonary disease or bacteremia (3–5, 7). The organism has been detected in blood cultures with broth systems, but prolonged incubation is required (5). Broth cultures in our system required 7 days or longer to become positive, whereas the L-C method detected the organism in less than 2 days.

The data from this case suggest that the L-C blood culture technique can be a sensitive, efficient, and rapid method for the detection and isolation of rapidly growing mycobacteria from blood and that quantitation with this method can be useful for monitoring antimicrobial therapy.

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