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Antibacterial Activity in Blood Cultures

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A total of 2,010 blood samples inoculated into tryptic soy broth were examined for antibacterial activity by means of a bioassay plate seeded with *Bacillus subtilis* spores. The size of the zone of inhibition on this plate was indicative of the degree of antibacterial activity. Current antibiotic therapy was confirmed by examination of chart records. Of the 2,010 blood cultures tested, 147 (7.3%) contained detectable levels of antibiotics; of these 147, 14 (9.5%) yielded growth of bacteria, and 133 (90.5%) remained negative. When the Antibiotic Removal Device (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.) was used, it eliminated the antibacterial activity but did not improve the recovery of bacteria from these cultures. Only bacteria resistant to the respective antibiotic were recovered from blood cultures that showed high levels of antibacterial activity (beta-lactam antibiotics, >0.60 μg/ml; aminoglycosides, >2 μg/ml; and tetracycline, >4 μg/ml). Blood cultures showing low levels of antibacterial activity yielded both resistant and susceptible bacteria.

Blood samples for culturing are sometimes drawn from patients who are receiving antimicrobial therapy (5). It has been shown that drugs can be transferred along with blood into the culture broth and suppress the growth of bacteria (10). Several methods have been developed to lessen this effect: substantial dilution of blood with broth (1), removal of microorganisms by membrane filtration (12), and inactivation of antimicrobial agents by penicillinase (3), sodium polyanetholesulfonate (13), or thiol broth (Difco Laboratories, Detroit, Mich.) (9). Two devices for removing antimicrobial agents, the Antibiotic Removal Device (ARD) (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.) and the BACTEC 16-B blood culture bottle (Johnston Laboratories, Inc., Towson, Md.), are also available.

In this study, we examined blood cultures for the presence of antibacterial activity and growth of microorganisms.

Blood samples (20 ml) were inoculated as follows: 10 ml into a bottle containing 50 ml of thioglycolate broth (Difco) with 0.025% sodium polyanetholesulfonate and 10 ml into a bottle containing 50 ml of tryptic soy broth with 0.025% sodium polyanetholesulfonate under vacuum with CO_2. Only the inoculated tryptic soy broth was examined for antibacterial activity. After 24 h of incubation, the blood culture bottles were processed for the isolation of bacteria by a conventional method (14). Testing for antibacterial activity was performed upon receipt of the blood cultures in the laboratory. A total of 2,010 blood cultures were monitored for 6 months as follows.

Mueller-Hinton agar bioassay plates seeded with *Bacillus subtilis* spores were prepared as described by Edberg and Sabath (4) and Sabath et al. (11). A total of seven wells were cut in the agar (2), and 0.05 ml of blood culture was inoculated into each well in the bioassay plate and incubated at 37°C for 8 h. The formation of a zone of inhibition around the inoculated well was indicative of antibacterial activity in the blood culture. Current antimicrobial therapy was confirmed by examination of the patient's chart. Blood cultures showing antibacterial activity were processed the next day with the ARD (6) as follows. Ten milliliters of the blood culture was introduced into the ARD and shaken on a vertical rotator (Marion Scientific) for 15 min. Each of two wells in the bioassay plate was inoculated with 0.05 ml of the suspension from the ARD to determine the effectiveness of antibiotic removal. The remaining broth was inoculated into a bottle containing tryptic soy broth and observed for 7 days along with the original blood culture.

Bacteria obtained from the cultures processed by the conventional method or with the ARD were identified and tested for antimicrobial susceptibility as described by Lorian and Waluschka (7) and Lorian et al. (8).

Standard antimicrobial solutions were tested on a bioassay plate at concentrations ranging from 5 to 0.2 μg/ml (4). Because antibacterial activity in the blood cultures were often caused by a combination of two or more agents, three individual antimicrobial standards were plotted on the same graph, and the degree of antibacterial activity was classified as low, medium, or high, depending on the zone size. The following levels of antibacterial activity were observed in the blood culture bottles when compared to the respective standards.

Low antibacterial activity was indicated by a zone of inhibition with a diameter of 8 to 12 mm, which corresponded to <0.25 μg/ml for beta-lactam antibiotics, <0.50 μg/ml for aminoglycosides, and <2 μg/ml for tetracycline.

Medium antibacterial activity was indicated by a zone of inhibition with a diameter of 13 to 18 mm, which corresponded to ≤0.60 μg/ml for beta-lactam antibiotics (except penicillin [≤0.15 μg/ml]), ≤2 μg/ml for aminoglycosides (except gentamicin [≤1 μg/ml]), and ≤4 μg/ml for tetracycline.

High antibacterial activity was indicated by a zone of inhibition with a diameter of >19 mm, which corresponded to >0.60 μg/ml for beta-lactam antibiotics (except penicillin [>0.15 μg/ml]) >2 μg/ml for aminoglycosides (except gentamicin [>1 μg/ml]), and >4 μg/ml for tetracycline.

Because blood was diluted 1/5 in broth, the actual antibacterial activity in the blood samples was approximately six times higher than in the respective blood cultures.

Of the 2,010 blood cultures tested, 147 (7.3%) showed antibacterial activity. These cultures did not show any level of antibacterial activity after being processed with the ARD. Organisms were recovered from 14 (9.5%) of these 147 blood cultures (12 patients) showing antibacterial activity. Further dilution of these positive blood cultures with the ARD and

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subsequently with tryptic soy broth did not affect the recovery of microorganisms. The same bacteria were also isolated by the conventional method. Bacteria, including organisms considered contaminants, were isolated from 328 (17.6%) blood cultures that did not show antibacterial activity. Antimicrobial therapy recorded in the charts of the above mentioned 12 patients when the blood samples were taken included one or more of the following antibiotics: ampicillin, penicillin, cephalothin, amikacin, gentamicin, and tetracycline.

The organisms recovered from the 14 blood cultures showing antibacterial activity included the following: one *Escherichia coli* strain susceptible to ampicillin, one *Staphylococcus aureus* strain susceptible to tetracycline, and one *Serratia liquefaciens* strain resistant to cephalothin recovered from blood cultures showing low levels of antibacterial activity; two *E. coli* strains, one susceptible to amikacin and the other resistant to ampicillin, two *S. marcescens* strains susceptible to gentamicin, one group B *Salmonella* strain susceptible to ampicillin, and one *S. aureus* strain resistant to penicillin recovered from blood cultures showing medium levels of antibacterial activity; and two *Pseudomonas aeruginosa* strains and one *S. epidermidis* strain resistant to gentamicin and two *P. aeruginosa* strains resistant to cephalothin recovered from blood cultures showing high levels of antibacterial activity. The levels of antibacterial activity in the 133 (90.5%) negative blood cultures were as follows: 27 with low levels, 61 with medium levels, and 15 with high levels.

Blood cultures with antibacterial activity showed a lower recovery rate of bacteria (9.5%) than positive blood cultures without antibacterial activity (17.6%) ($0.01 < P < 0.05$).

Three phases of bacterial suppression in blood samples obtained during the course of antimicrobial therapy have been recognized: complete, partial, and none (10). In this study, the recovery of bacteria from blood could be divided into two categories related to the level of antibacterial activity: first, organisms that were susceptible or resistant to antimicrobial agents and that were recovered from blood cultures showing low or medium levels of antibacterial activity, and second, organisms that were resistant to antimicrobial agents and that were isolated from blood cultures showing high levels of antibacterial activity. It appears, therefore, that a high level of antibacterial activity in a blood culture can prevent the growth of organisms susceptible to the antimicrobial agent administered, whereas medium and low levels of antibacterial activity do not preclude the isolation of antibiotic-susceptible organisms. The ARD removed the antibacterial activity from all 147 blood cultures but did not lead to the recovery of organisms from 133 of these 147 blood cultures which were negative before the ARD was used. All 14 blood cultures positive after the ARD was used were also positive when the conventional system was used. This suggests that the antibacterial activity leading to negative blood cultures actually took effect either in the patient or during the first 24 h of incubation of the blood culture.

To improve the recovery of bacteria from blood cultures while a patient is receiving antimicrobial therapy, blood samples should be taken when the antimicrobial concentrations are expected to be at their lowest level, i.e., just before the administration of the next dose.

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