

Rapid Isolation of Bacteria from Septicemic Patients by Use of an Antimicrobial Agent Removal Device

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The new Antimicrobial Removal Device increased the efficacy of conventional methods for isolating bacteria from the blood of septicemic patients. The device removes as much as 100 μ g of antibiotics per ml from whole blood without a significant decrease in bacteria. Of 51 patients studied, 31 yielded positive cultures, and the Antimicrobial Removal Device permitted earlier isolation of the causative agent from blood. Subcultures within the first 12 h yielded 12 positive isolates, as compared to 2 when subcultured without processing in the device. Overall, 21 of the 31 agents were isolated more rapidly after Antimicrobial Removal Device treatment, and four samples were positive only after such treatment. One agent was not isolated in the treated culture.

Despite the availability of antimicrobial drugs, the mortality rate from bacteremia remains high. The identification and susceptibility of the infecting organism should be determined as early as possible in the course of bacteremia, since the early administration of the appropriate antibiotic greatly improves the chances of survival of the patient (5). However, rapid isolation of the offending organisms from bacteremic patients may be difficult when the patient has been administered antibiotics, which are transferred along with the bacteria in the blood into the culture broths and often inhibit the growth of the agent (1, 2, 4). Natural opsonins, beta-lysins, and other inhibitors in blood also suppress bacterial growth in cultures.

Recently, Melnick and Wallis (J. L. Melnick and C. Wallis, U.S. Patent 4,145,304, 1979) developed a method for the removal of antibiotics and other bacterial inhibitors from whole blood without significantly decreasing the bacterial titer. They designed a mixed-resin system called the Antimicrobial Removal Device (ARD), which efficiently removes antibiotics and some bacterial inhibitors from whole blood after tumbling the mixture under specific conditions. The current report is concerned with the clinical application of the ARD as compared to conventional blood culturing to determine its efficacy in isolating bacteria from septicemic patients.

MATERIALS AND METHODS

ARD. ARDs were provided by Marion Laboratories Inc., Kansas City, Mo. Preparation of these devices has been described in detail (Melnick and Wallis, U.S. Patent 4,145,304, 1979). In brief, the ARD consists of a 60-ml rubber-capped vial loaded with 10 g of cationic resin (C249-Ionac, Birmingham, N.J.) and 15 g of

polymeric adsorbant resin (Amberlite XAD₄, Rohm & Haas, Philadelphia, Pa.), which have been pre-treated to prevent bacterial retention. The 25 g of mixed resin is fluidized with 5 ml of physiological saline. The inner portion of the rubber cap used to seal the ARD is equipped with a filter guard that prevents resin from entering the needle when fluids are removed from the device. The sealed device is then sterilized by autoclaving. The complete ARD bottle is available from Marion Laboratories (Kansas City, Mo.). For use, 5 to 10 ml of whole blood is aseptically injected into the ARD, which is then placed on a tumbler (Applied Poly-Technology, Inc., Houston, Tex.) specifically designed for rotating the ARD on its axis at 84 rpm. This rotation maintains the resin in suspension for maximum removal of antibiotics. After 15 min of tumbling, the ARD is removed from the tumbler, and the blood-saline mixture is aseptically withdrawn and transferred to a standard blood culture broth.

Patients. The patients in this study had a history of recent or current antibiotic therapy and were thought to be septicemic. Antibiotic blood levels were taken from the hospital record. Blood cultures had been ordered on all patients by their physicians as an aid to diagnosis.

Blood cultures and experimental procedures. Blood in 15-ml samples was aseptically collected by syringe. One 5-ml sample (the first) was transferred to a blood culture broth (Lederle), and was examined according to routine hospital practice. Another 5-ml sample (the second) was added to a duplicate blood culture broth, from which samples were later subcultured as indicated. The last 5-ml sample (the third) was inoculated into the ARD at bedside and immediately taken to the laboratory, where it was tumbled for 15 min. The supernatant mixture of the third bottle was then aspirated and aseptically transferred to a standard blood culture broth. The three blood cultures from the same patient were then incubated at 35°C, with subcultures performed from the second and third

bottles at 3, 8, 24, 48, and 72 h and at 7 days. The experimental procedures and results on the samples used for the second and third cultures had no influence on patient therapy. Our study compares the ability and time to isolate organisms in the second (standard) and third (ARD) blood culture broths.

RESULTS

Fifty-one patients were evaluated, of whom 31 proved to be bacteremic. Earlier isolation of bacteria from blood occurred in 21 of the matched cultures when the blood was first processed by the ARD as compared to standard cultures. In four instances, bacteria were recovered only from the ARD-processed samples, whereas only once was the infectious agent recovered only in the standard culture.

As noted in Table 1, 13 positive subcultures were obtained when planted within the first 12 h after the ARD treatment, but only 2 were obtained by the standard blood culture method within the same time period. The three cases that were positive by subculture after 7 days of incubation in the standard culture group were organisms (two *Staphylococcus aureus* and one *Bacteroides* strains) that would have been expected to grow readily in the culture broth before the 7-day period. However, the high concen-

tration of antibiotics in these patients delayed growth of the organism even when the culture was diluted by subculturing. The two *S. aureus* patients had blood levels of 4.5 μg of tobramycin and 10 μg of gentamicin per ml, respectively, and the patient with *Bacteroides* had 5 μg of amikacin per ml, concentrations that are quickly removed by the ARD before the blood is cultured.

Table 2 shows the results of the four subcultures that were positive using the ARD and negative by standard cultures, and the one subculture that was positive by the standard culture method but negative by ARD. Patient no. 8 had a serum level of 8.1 μg of tobramycin per ml; its removal by the ARD allowed isolation of *S. aureus* from the blood subculture taken at 8 h. Similar findings were obtained in patient no. 43, who had a serum level of 3.3 μg of gentamicin per ml. Patients no. 35 and 38 were not tested for serum antibiotics. One had septicemia with *Klebsiella pneumoniae*, and the other had septicemia with *Neisseria gonorrhoeae*, which were isolated in 24- and 48-h subcultures by the ARD only.

The frequency of distribution of infectious agents isolated in this study (strain and number of cases) was as follows: *Escherichia coli*, 4; *Providencia stuartii*, 1; *Serratia marcescens*, 1; *Streptococcus epidermidis*, 2; *S. aureus*, 10; *Enterobacter aerogenes*, 2; *Streptococcus pneumoniae*, 2; *Clostridium perfringens*, 1; *Proteus mirabilis*, 1; *K. pneumoniae*, 2; *N. gonorrhoeae*, 1; *Streptococcus* group A, 1; *Bacteroides thetaiotaomicron*, 1; *Bacteroides fragilis*, 1; *Bacteroides melaninogenicus*, 1; *Bacteroides ovatus*, 1; *Bacillus cereus*, 1; *Propionibacterium acnes*, 1; mixed infections, four pairs.

DISCUSSION

Antimicrobial therapy may often be initiated as "broad" coverage, particularly if the situation is life threatening. This broad coverage is not

TABLE 1. Comparison of subcultures taken at intervals from standard blood cultures and from those with prior ARD processing^a

Culture method	No. of subcultures first positive at time:							No. with all subcultures negative (3 to 168 h)
	3 h	8 h	12 h	24 h	48 h	72 h	168 h	
Standard blood culture	0	0	2	13	13	2	3	4
Prior ARD processing	1	6	6	15	6	2	0	1

^a The data were collected from samples taken from 31 patients who yielded one or more positive cultures.

TABLE 2. Cultures that proved positive with one system and negative by the comparative system

Patient no.	Diagnosis	Antibiotic and serum level ($\mu\text{g}/\text{ml}$)	Bacteria isolated	Recovery time	
				ARD	Conventional
8	Septicemia	Tobramycin (8.1)	<i>S. aureus</i>	8 h	Neg.
22	Burns	Gentamicin (0.5) Amikacin (5.0)	<i>S. aureus</i>	Neg	7 days
35	Fever	Not done	<i>K. pneumoniae</i>	24 h	Neg.
38	Pelvic inflammatory disease	Not done	<i>N. gonorrhoeae</i>	48 h	Neg.
43	Osteomyelitis	Gentamicin (3.1)	<i>S. aureus</i>	12 h	Neg.

dependent on the isolation of the causative organisms.

If the organism is isolated, antibiotic susceptibility testing can be performed, and definitive therapy can be initiated. In septicemia, isolation from the blood is often slowed down or completely prevented by the early administration of inappropriate or inadequate therapy. The ARD is specifically designed to remove "shotgun" antibiotics from the blood sample before testing to allow increased isolation and earlier isolation than current methodology. Before the trial reported here, a large number of antibiotics were tested (Melnick and Wallis, U.S. Patent 4,145,304, 1979), and all, up to 100 μ g, were found to be rapidly and completely removed from the blood by the ARD and tumbler described in the present paper. In the clinical study reported here, the device performed well; earlier isolation was found in 21 of 30 patients, and in 4 patients isolation occurred only with ARD-processed blood. In only one instance was a blood culture positive with standard technique and not in the ARD-processed blood.

The positive results of this study agree with a subsequent study on *Staphylococcus endocarditis*, which showed that in 47 cultures from patients on antibiotic therapy, 4 were positive both by standard blood culturing and after ARD treatment, but 22 were positive only when the blood was processed by the ARD (J. A. Washington II, Mayo Clinic, personal communication).

The separation of bacteria from antibiotics in blood by membrane filtration (5) is time con-

suming, and the manipulations seem conducive for contamination. Higher efficiency of isolations should be achieved by using the ARD in conjunction with the BACTEC system (3). The uptake of radioactive materials in this test by bacteria is also delayed by the presence of antibiotics in blood, since metabolism of labeled dextrose or amino acids is inhibited when bacteria are not biosynthetically active. Thus, the use of the ARD should enhance the ability and time of diagnosis of septicemia by BACTEC as well as by standard methods.

ACKNOWLEDGMENTS

We thank Linda M. Ribarich for expert technical assistance and John A. Washington II (Mayo Clinic) for permission to refer to unpublished data on the use of the ARD for rapid detection of bacteremia in antibiotic-treated patients.

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