Evaluation of the Antibiotic Removal Device

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A new system called an Antibiotic Removal Device (ARD), used for the removal of residual antibiotics in blood specimens, was studied in 300 patients diagnosed as clinically septic despite antimicrobial therapy. Blood specimens from these patients were processed with and without the ARD into aerobic and anaerobic media. There were 53 patients who had one or more positive blood culture bottles, for a total of 109 positive blood culture bottles. Of these 109 bottles, 33.9% were positive with ARD-processed blood in aerobic media, 24.8% with ARD-processed blood in anaerobic media, 22.9% with conventionally processed blood in aerobic media, and 18.3% with conventionally processed specimens in anaerobic media. After 6 h of incubation, 14 bottles with blood processed by the ARD and 9 bottles processed conventionally were positive; after 24 h, 48 bottles with blood processed by the ARD and 31 bottles processed conventionally were positive. The same organisms were isolated from both ARD- and conventionally processed blood, with four exceptions. The ARD system, when compared with a conventional system of blood specimen processing, significantly increased the detection of bacteremia and decreased the time required for its detection in patients clinically septic despite ongoing antimicrobial therapy.

Current emphasis in clinical microbiology is on the development of rapid, easy methods which will provide results that have clinical significance. The need for such methodology is especially true for the detection of bacteremia in patients already receiving antimicrobial agents (1). Because of the potential seriousness of the syndrome, patients suspected of being septic are often given antibiotics before blood cultures are obtained, thus reducing the incidence of the recovery of bacteria (1). In addition, patients receiving antibiotics for prophylaxis may become septic while on therapy. Increasing the yield of positive blood cultures in both of these groups of patients would allow for a more rational use of antibiotics.

The Antibiotic Removal Device (ARD; Marion Laboratories, Inc.) is a new system for removing residual antibiotics from blood specimens. This study was designed to determine the effectiveness of the ARD in the recovery of bacteria from blood specimens of patients receiving antimicrobial therapy and to compare the ARD with a conventional system of culturing blood.

MATERIALS AND METHODS

Selection of study patients. Patients were selected for the study if their primary care physicians considered them to have clinical signs of sepsis and continuing infection despite antimicrobial therapy. All patients were receiving at least one, and most were receiving two, antibiotic drugs at therapeutic doses.

Collection and processing of specimens. Protocol called for 20 ml of blood to be drawn by syringe from each patient by a nurse. At the bedside, 4 ml of blood was put into each of two ARD bottles, into one aerobic blood culture bottle, and into one anaerobic blood culture bottle. The aerobic blood culture medium consisted of 30 ml of tryptic soy broth (BACTEC 6) with 0.025% sodium polyanethol sulfonate, and the anaerobic culture medium consisted of 30 ml of preduced, anaerobically sterilized tryptic soy broth (BACTEC 7) with 0.025% sodium polyanethol sulfonate. The remaining specimen was centrifuged, and the serum was separated and frozen for future studies.

The two ARD bottles and the two culture bottles were taken back to the laboratory, where the ARD bottles were shaken for 15 min as directed by the manufacturer. Then, all of the liquid from the bottles was removed aseptically with syringes and transferred to aerobic or anaerobic tryptic soy broth. Thus, for each blood specimen, two bottles of aerobic medium, referred to as O2 bottles, and two bottles of anaerobic medium, referred to as AnaO2 bottles, were inoculated. One aerobic and one anaerobic bottle contained blood which had been processed through the ARD. These were referred to as the ARD set. One aerobic and one anaerobic bottle contained blood which was inoculated directly. These two bottles served as controls and were referred to as the conventional set.

Both sets were incubated in a shaker incubator at 35°C for the first 24 h of an incubation period of 14 days.

Detection of positive blood cultures. To detect microbial growth in the cultures, we routinely subcultured after incubation periods of 6, 24, and 48 h and 7 and 14 days. In addition, whenever a bottle was visibly turbid, it was subcultured. In the ARD-processed specimens, turbidity was occasionally difficult to assess.
because the blood cells were lysed. The bottles were also checked by the BACTEC radioisotope-labeled substrate system at 6 h and 1, 2, 7, and 14 days and subcultured if an appropriate increase in radioactive-labeled gas was noted.

The subculturing was done to two blood agar plates and one chocolate plate. The first blood agar plate was incubated anaerobically by using a GasPak system (BBL Microbiology Systems), the second blood agar plate was incubated aerobically, and the chocolate plate was incubated with 10% carbon dioxide.

Identification of blood cultures. Aerobic and facultatively anaerobic organisms were identified by the methods described in the Manual of Clinical Microbiology (5). Anaerobic bacteria were identified with methods described in the Wadsworth Anaerobic Bacteriology Manual (7) and the VPI Anaerobe Laboratory Manual (2).

Statistical analysis was performed with a nonparametric test for matched-pair analysis of discrete data (McNemar test).

RESULTS

Three hundred patients were included in this study. Aminoglycoside serum levels were measured in study patients with and without positive blood cultures and were not found to differ significantly between the two groups. Not all patients had a large enough specimen drawn for all four blood culture bottles. A total of 296 aerobic bottles and 254 anaerobic bottles were inoculated with blood that had been processed through an ARD. A total of 297 aerobic and 259 anaerobic bottles were inoculated with blood processed in a conventional manner. These were referred to as Conv-O2 and Conv-AnO2 bottles.

Positive blood cultures. There were 109 positive blood culture bottles. All positives occurred in bottles for which there was a corresponding control or ARD set. Of the 109 positive bottles, 37 (33.9%) ARD-O2 bottles, 27 (24.8%) ARD-AnO2 bottles, 25 (22.9%) Conv-O2 bottles, and 20 Conv-AnO2 (18.3%) bottles were positive (Table 1). The difference in recovery for bacteremic patients between ARD- and conventionally processed bottles was statistically significant (P < 0.01).

Of the 300 patients, 53 (17.6%) had positive blood cultures. Of these 53 patients, (20/38%) had positive cultures only in those bottles with blood processed with the ARD. Of the 53 patients, 7 (13%) had positive cultures only in specimens processed in the conventional manner. This difference is statistically significant (P < 0.02). Of the 53 patients, 26 (49%) had positive cultures in bottles processed with both the ARD and the conventional method. The overall differences between the two processing systems is statistically significant (P < 0.01).

Time to positivity. We examined the time interval between drawing the blood cultures and their detection as positive, or at their "time to positivity," a term used by Ilstrup (3). The time to positivity of the majority of the bottles was 48 h of incubation (Fig. 1). After 6 h of incubation, 10 ARD-O2 bottles, 4 ARD-AnO2 bottles, 6 Conv-O2 bottles, and 3 Conv-AnO2 bottles were positive, as detected by blind subculturing. None of the bottles appeared to be turbid, and only one bottle had a BACTEC reading of greater than 30 BACTEC units by 6 h of incubation. The difference in time to positivity between the two processing techniques was statistically significant at 24 h and thereafter (P < 0.01).

Bacteria isolated. There were 48 isolates from the ARD-processed specimens and 35 isolates from the conventional specimens (Table 2). All species of organisms, except four, were isolated from both the ARD- and conventionally processed blood, although not in equal numbers. For example, although Staphylococcus aureus was isolated by both systems, it was isolated from 10 ARD-processed specimens and from 2 conventionally processed specimens. The difference in the isolation of S. aureus from patients with positive cultures between the two systems was significant (P < 0.02). Escherichia coli was isolated from nine ARD-processed specimens and from four conventionally processed specimens. This difference in isolation of E. coli was not significant (P > 0.1; P < 0.2). The difference between the two systems in isolation of organ-

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**TABLE 1. ARD versus conventional positive blood culture bottles**

<table>
<thead>
<tr>
<th>Media</th>
<th>No. of positive/total specimens processed by:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ARD (%)</td>
<td>Conventional method (%)</td>
</tr>
<tr>
<td>Aerobic</td>
<td>37/52 (71.2)</td>
<td>25/52 (48.1)</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>27/52 (51.9)</td>
<td>20/52 (38.5)</td>
</tr>
</tbody>
</table>

**FIG. 1.** Interval between the time the blood cultures were drawn and their detection as positive.
isms other than *S. aureus* was not significant (*P* > 0.2). However, if isolates of *Staphylococcus epidermidis* and *Propionibacterium acnes* are excluded, as these are often considered contaminants, then the difference in isolation of pathogens other than *S. aureus* is significant (*P* < 0.02).

**Occurrence of contaminants.** Since blood processed with an ARD requires more handling, we feared that these specimens would be contaminated more frequently. However, two organisms that are often considered contaminants were isolated infrequently. Coagulase-negative staphylococci were isolated from three ARD bottles and from seven conventional bottles. *P. acnes* was isolated from blood processed by the conventional manner.

**DISCUSSION**

Patients with septicemia are those most seriously ill, and positive blood cultures are often the results of highest clinical significance that a microbiology laboratory can report. Because of the serious outcome of a diagnosis of septicemia, many patients receive antimicrobial therapy as soon as the diagnosis is made and before blood cultures or blood culture results are obtained. The detection of bacterial growth in blood cultures from these patients can be prevented or delayed by antimicrobial therapy. Prior solutions to this problem have been dilution of the blood and antimicrobials in broth; inactivation of residual antimicrobial agents with such additives as penicillinase, *para*-aminobenzoic acid, or sodium polyanethol sulfonate; and removal of the organism by membrane filtration (6). The ARD offers a new approach for the removal of residual antimicrobial agents (8).

We studied the ARD in patients who were diagnosed as septic, were receiving antibiotic therapy, and continued to have clinical signs of sepsis. All patients were receiving at least one, and, in most cases, two antimicrobial agents at customary therapeutic doses. The ARD significantly increased the detection of bacteremia as compared with conventional processing in this group of patients. Moreover, the time to detection of a positive culture was shorter for ARD-processed specimens than for conventionally processed specimens.

The same organism could be isolated from both ARD- and conventionally processed blood, with four exceptions. The increased specimen handling involved in ARD processing did not increase the isolation rates of organisms which could be considered contaminants, i.e., *P. acnes*, *S. epidermidis*, and *Bacillus* sp.

Kunin et al. (4) have reported that between 27 and 29% of the patients admitted to their hospital were treated with antibiotics. On surgical services, 58% of these courses were for prophylactic indications. It can be reasonably argued that the ARD would be of value in detecting bacteremias in this large group of patients should clinical signs of sepsis occur during or shortly after such therapy. Surveys of antibiotic usage at our hospital indicate that between 50 and 80% of the patients in intensive care units are receiving antibiotics. Many of the patients in this study were drawn from that population, and we consider the ARD an important asset in the detection of pathogens in those patients with
continued signs of sepsis. Rationalization of antibiotic therapy based on such detection, though not studied prospectively by us, would appear to be a major benefit.

We conclude that the ARD was of considerable value in increasing the detection of microbial growth in blood specimens obtained from patients who were septic despite ongoing antimicrobial therapy. Not only did ARD processing significantly increase the detection of bacteremias, but it also decreased the time required for the detection of positive blood cultures. These findings indicate an important role for this clinically useful device.

LITERATURE CITED