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Controlled Evaluation of Modified Radiometric Blood Culture Medium Supplemented with Gelatin for Detection of Bacteremia and Fungemia

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Although the addition of 1.2% gelatin to broth blood culture media containing sodium polyanetholesulfonate has been shown to enhance detection of certain bacteria, including *Neisseria meningitidis*, *N. gonorrhoeae*, *Peptostreptococcus anaerobius*, and *Gardnerella vaginalis*, the effect of such supplementation on the detection of other microorganisms causing bacteremia and fungemia is not known. Therefore, we studied BACTEC 6B medium with and without gelatin in 6,833 paired comparisons to examine the effects of supplementation on both the yield and the speed of detection of sepsis. More aerobic and facultative bacteria grew in the 6B than in the 6B-gelatin medium ($P < 0.001$), especially staphylococci ($P < 0.01$), *Escherichia coli* ($P < 0.01$), other members of the family *Enterobacteriaceae* ($P < 0.05$), and *Acinetobacter* spp. ($P < 0.05$). When microorganisms grew in both bottles, they did so earlier in 6B than in 6B-gelatin ($P < 0.001$). We conclude that the 6B medium in its present formulation is superior to 6B medium supplemented with 1.2% gelatin.

The anticoagulant sodium polyanetholesulfonate (SPS) now is added routinely to virtually all commercial blood culture media (10). The beneficial effects of this anticoagulant are well documented and include inhibition of phagocytosis and complement and lysozyme activities as well as inactivation of aminoglycoside antibiotics (1, 10, 14). Also well documented are the inhibitory effects of SPS on the growth of *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Peptostreptococcus anaerobius*, *Streptobacillus moniliformis*, and *Gardnerella vaginalis* (2, 4, 5, 9, 12). Several investigators have shown that the inhibitory effects of SPS on bacterial growth can by countered by supplementing blood culture media with 1.2% gelatin (3, 8, 9, 11, 17). However, the effect of the addition of gelatin to blood culture broth on the overall detection of bacteremia and fungemia has never been studied in a large field trial. Therefore, we compared the yield and speed of detection of clinically important microorganisms from 6,833 blood cultures at five hospitals that use identical radiometric methods for culturing blood.

MATERIALS AND METHODS

Collection of samples. During the study period, three 30-ml BACTEC bottles (aerobic 6B, aerobic 6B with 1.2% gelatin [prepared by Johnston Laboratories], and anaerobic 7D) containing tryptic soy broth with 0.025% SPS were used for all blood cultures from adult patients at Robert Wood Johnson University Hospital, the University of Colorado Hospital, the Denver Veterans Administration Medical Center, the Salt Lake City Veterans Administration Medical Center, and Vanderbilt University Medical Center. Patient blood cultures were obtained at bedside after preparation of the skin with 10% povidone-iodine (1% available iodine) followed by 70% isopropyl alcohol. Blood from each separate venipuncture was distributed by needle and syringe as follows: 5 ml to BACTEC 6B bottle, 5 ml to BACTEC 6B with gelatin bottle, and 5 ml to BACTEC 7D bottle.

Volume standards. To ensure that the culture bottles actually received the specified amounts of blood, we measured the level of fluid in each container after it was filled with blood. Although all blood-containing bottles were incubated, those with fluid levels below the standards were coded as inadequate and were excluded from subsequent analyses. Fluid level standards were set to ensure that at least 4 ml was added to each bottle.

Processing of samples. Identical methods were used for processing the blood cultures in the clinical microbiology laboratories at all five hospitals. All bottles were incubated in an air incubator at 35°C for 7 days. The 6B and 6B with gelatin bottles were examined macroscopically and radiometrically twice daily on days 1 and 2 of incubation and once daily thereafter through day 7. The BACTEC 7D bottle was examined macroscopically and radiometrically once daily. The 6B and 6B with gelatin bottles were shaken for the first 24 to 48 h of incubation, whereas the 7D bottle was not. Gelatin-containing 6B and 6B without gelatin with a growth index of 30 or greater and 7D bottles with a growth index of 20 or greater were examined by Gram stain and subcultured. In addition, bottles with an increase of 10 or more growth index units between readings were examined by Gram stain and subcultured. All microorganisms isolated were identified by standard procedures (6).

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TABLE 1. Comparison of yield of clinically important bacteria and fungi from BACTEC 6B medium with and without 1.2% gelatin

Microorganisms	No. of isolates recovered by:			P
	Both media	6B only	6B-gelatin only	
Aerobic and facultative bacteria	403	114	51	<0.001
Gram positive	235	56	30	<0.01
Staphylococcus aureus	111	20	12	NS[a]
Staphylococcus epidermidis	42	15	3	<0.01
Streptococci	77	20	14	NS
Other[b]	5	1	1	NS
Gram negative	168	58	21	<0.001
Escherichia coli	56	22	7	<0.01
Other members of the family *Enterobacteriaceae*	72	17	6	<0.05
Pseudomonas aeruginosa	26	8	7	NS
Other[c]	14	11	1	<0.01
Anaerobic bacteria[d]	5	3	2	NS
Fungi[e]	32	17	15	NS

[a] NS, Not significant ($P > 0.05$).
[b] Includes *Bacillus* spp., 2; *Corynebacterium* spp., 3; and *Listeria monocytogenes*, 2.
[c] Includes *Acinetobacter* spp., 15; *Campylobacter* spp., 3; *Haemophilus influenzae*, 3; *Neisseria* sp., 1; *Eikenella corrodens*, 2; and *Aeromonas hydrophila*, 1.
[d] Includes *Clostridium perfringens*, 4; *C. sporogenes*, 2; and *Bacteroides fragilis*, 4.
[e] Includes *Candida albicans*, 41; *C. tropicalis*, 13; *C. parapsilosis*, 5; *Cryptococcus neoformans*, 4; and one unidentified yeast.

Clinical assessment. All patients with positive blood cultures were evaluated by an infectious disease specialist who defined pathogens (clinically important microorganisms causing sepsis) and contaminants by established criteria (16). An episode of bacteremia or fungemia was defined by the first positive blood culture or by a new positive blood culture result occurring more than 2 days after the preceding positive (unless it was obvious clinically that the new positive did not represent a new bacteremic event). Any positive culture obtained within 48 h of a previous positive was considered to represent the same episode (16).

Analysis of data. Paired comparisons of the 6B and 6B with gelatin bottles were done only on adequately filled bottles that grew microorganisms causing true bacteremia or fungemia. Significance testing was done with the modified chi-square test described by McNemar (7).

RESULTS

A total of 6,833 adequately filled blood culture sets were received during the study period. Of these, 769 (11.3%) were positive, including 589 (8.6%) that grew microorganisms causing illness, 102 (1.5%) that grew contaminants, 4 (0.1%) that grew one or more contaminants and pathogens, and 74 (1.1%) that grew microorganisms that were indeterminate as a cause of sepsis. A total of 642 isolates associated with sepsis were detected; 440 grew in both the 6B and gelatin-supplemented 6B bottles, and 343 of these were detected at the same time. A total of 134 (20.9%) isolates associated with sepsis grew only in the 6B medium, whereas 68 (10.6%) grew only in the gelatin-supplemented 6B medium.

More aerobic and facultative bacteria grew in the 6B medium than in the gelatin-supplemented 6B medium ($P < 0.001$; Table 1). In particular, the yield of *Staphylococcus epidermidis* ($P < 0.01$), *Escherichia coli* ($P < 0.01$), other members of the family *Enterobacteriaceae* ($P < 0.05$), and *Acinetobacter* spp. ($P < 0.05$) was better in the absence of gelatin supplementation. The yield of *Staphylococcus aureus*, streptococci, including enterococci, *Pseudomonas aeruginosa*, and yeasts was not different statistically in the two media, although trends favoring the 6 B medium without gelatin were noted for *Staphylococcus aureus* and streptococci. Contaminants, especially *Staphylococcus epidermidis*, also were isolated more frequently ($P < 0.01$) in the conventional 6B medium without gelatin.

When microorganisms grew in both media, they did so earlier in the 6B medium (Table 2). This was true especially for staphylococci ($P < 0.05$) and members of the family *Enterobacteriaceae* other than *E. coli* ($P < 0.05$).

DISCUSSION

Although the addition of gelatin to blood culture media containing SPS has been shown to improve the yield of *N. meningitidis* (2, 8), *N. gonorrhoeae* (12), *Peptostreptococcus anaerobius* (17), and *G. vaginalis* (9), the effect of gelatin supplementation on the detection of many common microorganisms causing bacteremia and fungemia has not been studied in a large field trial. Therefore, we evaluated the widely used BACTEC 6B medium, which contains tryptic soy broth supplemented with SPS, in its currently available formulation with the same medium that differed only by the addition of 1.2% gelatin.

The results clearly favored the current formulation, both for yield (Table 1) and for speed of detection (Table 2). Unfortunately, despite the large number of clinically significant positive blood cultures in this study, none of the microorganisms reported to be recovered with greater frequency in gelatin-supplemented media were detected. Whether this is due to the population of patients studied (all

TABLE 2. Comparison of speed of detection of clinically important bacteria and fungi from BACTEC 6B medium with and without 1.2% gelatin

Microorganisms	No. of isolates recovered by:			P
	Both media at same time	6B earlier	6B-gelatin earlier	
Aerobic and facultative bacteria	322	57	24	<0.001
Gram positive	182	35	18	<0.05
Staphylococci	116	26	11	<0.05
Streptococci	63	8	6	NS[a]
Other[b]	3	1	1	NS
Gram negative	140	22	6	<0.005
Escherichia coli	53	2	1	NS
Other members of the family *Enterobacteriaceae*	51	16	5	<0.05
Pseudomonas aeruginosa	23	3	0	NS
Other[c]	13	1	0	NS
Anaerobic bacteria[d]	0	2	3	NS
Fungi[e]	21	6	5	NS

[a] NS, Not significant ($P > 0.05$).
[b] Includes *Bacillus* spp., 2; *Corynebacterium* spp., 2; and *Listeria monocytogenes*, 1.
[c] Includes *Acinetobacter* spp., 6; *Aeromonas hydrophila*, 1; *Campylobacter jejuni*, 2; *Eikenella corrodens*, 2; *Haemophilus influenzae*, 2; and *Neisseria* sp., 1.
[d] Includes *Bacteroides fragilis*, 2; *Clostridium perfringens*, 1; and *C. sporogenes*, 2.
[e] Includes *Candida albicans*, 20; *C. tropicalis*, 7; *C. parapsilosis*, 3; and *Cryptococcus neoformans*, 2.

adults), the relative rarity of these organisms as causes of bacteremia, or the BACTEC 6B medium itself is not known.

It is not difficult to explain the contrast between our findings and those of Pai and Sorger (8), who noted that the addition of 1% gelatin to Columbia broth enhanced the detection of *N. meningitidis* but did not influence the recovery of other microorganisms from blood. There were multiple differences between that study and this one, including medium (Columbia broth versus tryptic soy broth), number of cultures studied (1,662 versus 6,833), patient population (pediatric versus adult), and number of positive cultures evaluated (45 versus 769).

An important role of SPS is the inactivation of complement (10). The countereffect of gelatin on SPS that allows growth of microorganisms such as *N. meningitidis* presumably is related to interference with complement inactivation by SPS. We speculate that the greater availability of complement in gelatin-supplemented media results in inhibition of serum-sensitive *Enterobacteriaceae* as well as other organisms (13, 15) and may explain both the lower yield (Table 1) and the slower growth (Table 2) of these organisms observed in our studies. There were too few of these other organisms in the studies of Pai and Sorger (8) to detect such differences. It is also possible that the effect of gelatin may be medium dependent. Most importantly, the effect of gelatin (or for that matter any additive) on any specific medium used for diagnostic studies in a clinical microbiology laboratory should be evaluated first in a large field trial and not be facilely assumed to be efficacious based on in vitro results with simulated cultures.

In summary, the currently marketed BACTEC 6B medium was shown to be superior overall to gelatin-supplemented 6B medium for both yield and speed of detection of microorganisms causing bacteremia and fungemia.

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