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Effects of Atmosphere of Incubation and of Routine Subcultures on Detection of Bacteremia in Vacuum Blood Culture Bottles

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Studies comparing isolation rates of bacteria and yeasts from vented and unvented vacuum blood culture bottles containing soybean-casein digest broth showed significantly more frequent and more rapid recovery of *Candida* and *Pseudomonas* from the vented bottle and no other statistically significant differences between the two. Subculture of bottles on the day of their collection was shown to accelerate recovery of 48% of positive cultures by day 1. A second subculture of known positive cultures yielded additional organisms in 1.5% of cultures.

Data obtained from studies of simulated blood cultures (5, 10) and of blood cultures in Columbia broth collected from patients with suspected bacteremia (3) have supported recently published recommendations that both aerobic and anaerobic blood cultures should be collected (1). Since many commercially prepared, conventional blood culture media are bottled under vacuum with CO_2 and since at least one such bottle has been shown to yield anaerobic bacteria at rates comparable to those obtained from prereduced anaerobically sterilized media (6, 13), it is possible to inoculate two bottles containing the same medium and to vent transiently only one of the two for purposes of aerobic culture. The second bottle is, therefore, not vented and is used for anaerobic culture.

In this study, we have examined the effects of venting on the recovery of bacteria and yeasts from a soybean-casein digest broth (tryptic soy, Difco Laboratories). Moreover, since it has been recommended that a routine ("blind") subculture be performed 1 day after collection of the blood culture (1), we have studied the value of an earlier routine subculture. Finally, we have resubcultured bottles known to contain microorganisms a week after their original subculture to determine the frequency with which additional organisms can be isolated.

MATERIALS AND METHODS

Blood collected aseptically by a venipuncture team from patients with suspected bacteremia was inoculated (10%, vol/vol) into each of two bottles containing 100 ml of tryptic soy broth under vacuum with CO_2 and with 0.025% sodium polyanetholsulfonate. Upon receipt of the bottles in the laboratory, one bottle was transiently vented with a sterile, cotton-plugged needle to release its vacuum. Both bottles were then incubated at 35 C and were examined macroscopically later on the day of their receipt, then once daily for 7 days, and finally on day 14 of incubation.

Routine subcultures of macroscopically negative bottles were performed by inoculating an aliquot of broth aspirated with a sterile needle and syringe onto chocolate blood agar plates which were incubated at 35 C in 10% CO_2. It has been customary in our laboratory in recent years to perform these subcultures 1 and 5 days after collection of the culture. For approximately 6 weeks during this study, an additional routine subculture was made on the day of each culture's collection.

Subcultures of positive or suspected positive cultures were made immediately to media appropriate for isolation of the microorganism seen in the Gram-stained smear. These subcultures routinely included a blood agar plate which was incubated anaerobically. With 393 positive cultures, an additional set of subcultures was made after 7 more days of incubation to ascertain the frequency with which additional species of bacteria would be isolated.

Methods of statistical analysis were based on those described by Cochran (4).

RESULTS

Between 1 October 1974 and 24 March 1975, 11,170 sets of blood cultures were collected. There were 1,160 positive cultures representing 572 patients. The number of isolates in positive cultures in vented and unvented bottles containing tryptic soy broth are listed in Table 1. Statistically significant differences between the vented and unvented bottles were limited to *Bacillus* ($P < 0.01$), *Pseudomonas* ($P < 0.05$), and *Candida* ($P < 0.001$) which were recovered more frequently from the vented bottle than

TABLE 1. *Numbers of isolates in tryptic soy broth: vented (V) and unvented (U)*

Organism	No. of isolates	No. of isolates in:			P value
		Both U and V	V only	U only	
Bacillus	28	0	22	6	0.01
Clostridium	8	4	0	4	NS[a]
Corynebacterium	160	23	58	79	NS
Escherichia	173	121	22	30	NS
Salmonella	20	17	2	1	NS
Citrobacter	10	9	0	1	NS
Klebsiella	66	43	16	7	NS
Enterobacter	14	12	1	1	NS
Serratia	12	8	4	0	NS
Proteus	30	13	8	9	NS
Eikenella	1	0	0	1	NS
Haemophilus	10	6	1	3	NS
Listeria	1	0	0	1	NS
Streptococcus					
Group A	10	7	1	2	NS
Group B	13	11	0	2	NS
Group D	56	51	1	4	NS
Group F	4	2	1	1	NS
viridans	108	84	11	13	NS
S. pneumoniae	58	50	4	4	NS
Other streptococci	23	19	1	3	NS
Eubacterium	1	0	1	0	NS
Acinetobacter	8	3	4	1	NS
Alcaligenes	5	1	2	2	NS
Moraxella	1	0	1	0	NS
Neisseria	5	0	4	1	NS
Bacteroidaceae	53	28	8	17	NS
Micrococcus	2	0	0	2	NS
Staphylococcus					
S. epidermidis	148	39	56	53	NS
S. aureus	120	85	13	22	NS
Peptostreptococcus	6	4	0	2	NS
Peptococcus	1	0	0	1	NS
Pseudomonas	67	40	20	7	<0.05
Aeromonas	3	3	0	0	NS
Candida	28	7	21	0	<0.001

[a] NS, Not significant.

from the unvented bottle. Although more clostridia and *Bacteroidaceae* were isolated from the unvented bottles than from the vented ones, the differences in their rates of isolation between the two bottles were not statistically significant. In those sets of cultures in which both the vented and unvented bottles were positive, *Pseudomonas* was recoverd, on the average, 1 day sooner ($P < 0.001$) from the vented bottle, and *Candida* was recovered, on the average, 4.71 days sooner ($P = 0.02$) from the vented bottle. There were no other statistically significant differences between the two bottles in terms of mean times to detection of positivity.

There were 128 positive cultures obtained during the early subculture study (Table 2). These bottles had been subcultured between 3 and 19.5 h (mean of 9.5 h) after their collection. Sixty-one (48%) were positive on the early subculture plates. An additional 59 (46%) were macroscopically positive after 48 h of incubation. Finally, of eight (6%) cultures which had remained macroscopically negative, seven were positive on the customary 24-h subculture plates, and one was positive on the customary 5-day subculture plates. Anaerobic bacteria and viridans streptococci were, with only one exception, never detected in the early subcul-

TABLE 2. *Means of detection of 128 positive blood cultures*

Cultures	Routine subculture (days)			Macroscopically positive	Total
	<1	1	5		
Number	61	7	1	59	128
Percent	48	5	1	46	100

ture. Conversely, 12 of 15 pneumococci were detected in the early subculture. There were no other types of bacteria predominantly detected by either method of subculture.

There were 393 positive cultures representing 241 patients which were resubcultured 7 days after their initial subculture to determine the frequency with which additional isolates could be recovered. Six additional isolates were recovered which had not been present in either the same bottles originally or in other blood cultures collected from the same patient within the same 24-h time interval. The six isolates were *Staphylococcus aureus* (four), *Streptococcus anginosus* (one), and *Klebsiella* (one).

DISCUSSION

Previously reported data from studies of simulated blood cultures by Knepper and Anthony (10) and by Gantz et al. (5) have shown that the growth of *Pseudomonas aeruginosa* and *Candida* spp. was inhibited in unvented vacuum blood culture bottles. Conversely, Gantz et al. (5) showed that the growth of *Bacteroides* was inhibited in vented vacuum blood culture bottles. These data were corroborated by Blazevic et al. (3) in a study of blood cultures collected from patients with suspected bacteremia. Significantly fewer isolates of *B. fragilis* and *Propionibacterium* were recovered from vented bottles containing Columbia broth, whereas significantly fewer isolates of *Pseudomonas, Candida, Cryptococcus neoformans,* and *S. epidermidis* were recovered from unvented bottles containing Columbia broth. Although differences in isolation rates between Columbia broth and tryptic soy broth have been shown to be limited to *S. aureus* and *Pseudomonas* (7), statistically significant differences between vented and unvented bottles containing tryptic soy broth were limited in our study to *Bacillus, Pseudomonas,* and *Candida*. Nonetheless, differences between studies notwithstanding, it appears to be necessary to use two vacuum blood culture bottles and to vent one bottle and not the other.

The value of routine ("blind") subcultures has been substantiated by Blazevic et al. (2) and by Hall et al. (7). It has rather arbitrarily been recommended that such subcultures be made 2 days and 5 to 7 days after venipuncture (1). The early subculture performed on the day of blood collection provided the initial means of detection of bacteremia and isolation of the etiologic agent in 48% of the positive cultures. We feel that the customary 24-h subculture simply postpones detection of those organisms not evident macroscopically for an additional day. With the remaining 46% which are initially detected macroscopically, subcultures are made to appropriate media for their isolation. As a result of these findings, we recommend that blood culture bottles be subcultured on the same day as they are collected. Because of practical considerations relating to transport time of the bottles to the laboratory and to bacterial growth characteristics, we arbitrarily elected not to subculture routinely in any less than 3 h after venipuncture. Cultures should then be examined macroscopically later in the day of their receipt and daily thereafter for 7 days and finally 14 days after collection. It is recommended that an additional routine subculture be made of macroscopically negative bottles 48 h after venipuncture.

Although polymicrobial bacteremia is known to occur (8, 9, 11, 12), its detection has usually been based upon the findings in the initial subcultures of a suspected positive culture. It was, therefore, of interest to us to resubculture cultures known to be positive after 1 week of additional incubation. Our findings demonstrating that additional organisms of possible clinical significance were recovered from 1.5% of positive cultures would hardly seem to justify the effort involved on a routine basis.

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