JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1983, p. 300–304
0095-1137/83/080300-05$02.00/0

Vol. 18, No. 2

Comparative Recovery of Bacteria and Yeasts from Lysis-Centrifugation and a Conventional Blood Culture System

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Received 21 March 1983/Accepted 29 April 1983

Blood cultures obtained with a lysis-centrifugation (L-C) system and a conventional two-bottle broth system were compared for the recovery of bacteria and yeasts from 7,000 cultures. The L-C system recovered significantly more total organisms, *Escherichia coli*, and *Candida* spp. and detected more patients with bacteremia and fungemia due to members of the family *Enterobacteriaceae* and yeasts. The broth system recovered significantly more streptococci and detected significantly more low-level *Pseudomonas* bacteremias. Polymicrobic bacteremia and fungemia were detected equally well by either culture system. Aerobic organisms grew equally well on blood, chocolate, or brain heart infusion agar plates used for L-C inoculation. A total of 82% of colony counts measured no more than 10 CFU/ml of blood, and it was at these low levels that enhanced detection of organisms by either system was observed. The L-C system isolated organisms and detected yeasts more rapidly than did the broth system. Contaminants occurred in 8.2% of L-C cultures and 1.9% of broth cultures. Low colony counts on L-C plates occurred for both *Staphylococcus epidermidis* contamination and septicemia.

A lysis-centrifugation (L-C) blood culture technique, which allows the direct plating of concentrated blood specimens onto agar plates, was first described and evaluated in 1976 (1, 2). An improved device was described in 1978 (4), and an evaluation in a clinical microbiology laboratory indicated that this method results in the increased isolation of *Staphylococcus aureus, Pseudomonas* spp., and yeasts when compared with a standard broth blood culture system (3). Since these three organisms are among the most frequent blood culture isolates in our hospital, we compared the L-C system with a conventional two-bottle broth method. We wanted to determine which system would: (i) recover more organisms, (ii) recover organisms more quickly, and (iii) provide isolated colonies more quickly. We also examined whether, by quantifying isolates, we could better determine when an organism such as *Staphylococcus epidermidis* was a contaminant.

MATERIALS AND METHODS

From September 1981 through September 1982, blood cultures obtained between 8 a.m. and 6 p.m. by the venipuncture team at Memorial Sloan-Kettering Cancer Center were entered into the study. These comprised 7,000 blood cultures out of a total of 21,000 obtained during this 12-month period. Equal amounts of blood ranging from 2 to 8 ml per tube were collected in an L-C tube (Isolator; E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) and a Vacutainer tube (100 by 16 mm) containing 5.95 mg of sodium polyanetholesulfonate (Becton, Dickinson & Co., Rutherford, N.J.). The L-C tube was inverted four times to ensure mixing, and both tubes were transported to the microbiology laboratory. Upon arrival in the laboratory, blood in the Vacutainer tube was inoculated equally into two bottles containing 50 ml of Columbia broth supplemented with cysteine (1 g/liter) (BBL Microbiology Systems, Cockeysville, Md.), and one of the bottles was transiently vented. Both bottles were incubated at 35°C and inspected for turbidity twice daily for 3 days and then daily for an additional 4 days. At 6 to 24 h, a "blind" subculture was performed on a chocolate agar plate, and this plate was incubated in 5% CO_2 in air at 35°C for 48 h. The L-C tube was processed according to the instructions of the manufacturer, in all cases, within 2 h of collection of the blood specimens. After lysis and centrifugation, the concentrated sediment remaining in the tube was divided equally and inoculated onto four agar plates. These included one Columbia sheep blood agar plate incubated anaerobically at 35°C for 4 days, one Columbia sheep blood agar plate and one chocolate agar plate incubated at 35°C in 5% CO_2 in air for 4 days, and one brain heart infusion agar plate incubated at 35°C in 5% CO_2 in air for 2 days and then at 30°C for an additional 4 days. The plates were inspected for visible colonies twice during day 1 and daily for the remainder of the incubation periods.

When growth of an organism was detected in the broth system, the bottle in which growth was seen, the time to first detection, and the time to first availability of isolated colonies were recorded. When growth of an

organism was detected on the plates inoculated from the L-C system, the time to first detection, the time to availability of isolated colonies, and the number of colonies per plate were recorded. CFU per milliliter of blood were also calculated. The identification of organisms was by standard methods.

Common skin and laboratory contaminants, including *Bacillus* spp., *Micrococcus* spp., diphtheroids, and molds, were considered contaminants unless clinical significance could be established by a review of laboratory and clinical information. The significance of *S. epidermidis* isolates was analyzed separately. The total number of positive blood cultures that each patient had for this organism, either in the study or from broth cultures that were obtained within 7 days before or after the L-C cultures were obtained, was determined. CFU in L-C cultures were compared, when available, for those patients with one, two, three, or four or more positive cultures for *S. epidermidis*.

The asymptotic chi-square test of McNemar (6), as applied to comparative blood culture methods by Ilstrup (5), was used for statistical analysis. Chi-square values of ≥ 3.84, which defined P values of ≤ 0.05, were considered significant.

RESULTS

From the 7,000 total cultures included in this study, 476 significant isolates were recovered from the L-C system or the broth system or both. The ability of each system to recover the species observed in this study is shown in Table 1. Significantly more total isolates ($P < 0.025$), *Escherichia coli* ($P < 0.005$), *Candida albicans* ($P < 0.05$), and *Candida glabrata* ($P < 0.025$) were isolated from the L-C system than from the broth system. Of the 476 significant isolates, 97 (20.4%) were from cultures with more than one significant organism. In these polymicrobic cultures, 75 organisms were recovered from each culture system, and neither system was significantly better for recovery of any of the organisms.

Since low numbers of some species were recovered, similar organisms were grouped for statistical purposes. The number of positive cultures and the number of patients with bacteremia or fungemia recognized by each system for

TABLE 1. Number of significant isolates from L-C and broth systems

Organism	Total no. of isolates	No. isolated[a] from:			P value
		L-C and broth	L-C only	Broth only	
Escherichia coli	102	57	32	13	<0.005
Staphylococcus aureus	57	38	13	6	
Klebsiella spp.	36	23	7	6	
Pseudomonas spp.	35	18	5	12	
Candida albicans	30	19	9	2	<0.05
Candida glabrata	29	17	10	2	<0.025
Streptococcus viridans	23	12	3	8	
Candida parapsilosis	19	9	8	2	
Bacteroides spp.	18	7	4	7	
Streptococcus spp. (group D)	17	10	4	3	
Enterobacter spp.	16	10	4	2	
Candida tropicalis	13	10	3	0	
Streptococcus pneumoniae	11	6	1	4	
Clostridium spp.	10	2	5	3	
Proteus spp.	8	3	4	1	
Corynebacterium sp. CDC-JK	8	1	2	5	
Serratia spp.	7	5	1	1	
Citrobacter spp.	6	3	2	1	
Acinetobacter spp.	5	1	3	1	
Streptococcus spp. (group B)	5	2	0	3	
Haemophilus spp.	3	0	0	3	
Listeria spp.	3	3	0	0	
Fusobacterium spp.	3	0	1	2	
Streptococcus spp. (group A)	2	2	0	0	
Streptococcus spp. (group G)	2	1	0	1	
Bacillus spp.	2	2	0	0	
Moraxella spp.	2	0	2	0	
Aeromonas sp.	1	1	0	0	
Providencia sp.	1	1	0	0	
Peptostreptococcus sp.	1	0	1	0	
Bifidobacterium sp.	1	0	0	1	

[a] Of the 476 positive isolates, 263 (55%) were isolated with L-C and broth, 124 (26%) were isolated with L-C only, and 89 (19%) were isolated with broth only ($P < 0.025$).

TABLE 2. Number of significant isolates and patients with bacteremia or fungemia recognized from L-C and broth systems

Organism or group	Total no. of isolates (patients)	No. of isolates (patients) recognized from:			P value for isolates (patients)
		L-C and broth	L-C only	Broth only	
Enterobacteriaceae	176 (130)	102 (71)	50 (41)	24 (18)	<0.005 (<0.005)
Yeasts	91 (40)	55 (24)	30 (14)	6 (2)	<0.0001 (<0.005)
Streptococcus spp.	60 (43)	33 (27)	8 (5)	19 (11)	<0.05 (NS[a])
Staphylococcus aureus	57 (41)	38 (26)	13 (11)	6 (4)	
Pseudomonas spp.	35 (24)	18 (10)	5 (5)	12 (9)	
Anaerobes	33 (26)	9 (8)	11 (5)	13 (9)	
Other	24 (18)	8 (7)	7 (5)	9 (6)	

[a] NS, Not significant.

these groups are shown in Table 2. Significantly more cultures that were positive for members of the family *Enterobacteriaceae* ($P < 0.005$) and yeasts ($P < 0.0001$) were recovered from the L-C system, and significantly more cultures that were positive for streptococci ($P < 0.05$) were isolated from the broth system. Significantly more patients with bacteremia and fungemia due to members of the *Enterobacteriaceae* ($P < 0.005$) and yeasts ($P < 0.005$) were detected with the L-C system. Of patients with *Enterobacteriaceae* bacteremia, 32% were recognized only with the L-C cultures. Of patients with fungemia, 35% were recognized only with the L-C cultures.

Table 3 shows the number of CFU per milliliter of blood as determined by L-C for each group of species and compares the ability of the two systems to detect bacteremia and fungemia according to colony counts. Cultures in which only the broth was positive were considered to contain less than 1 CFU/ml as measured by the L-C system. Overall, 52% of cultures contained less than 1 CFU/ml, and 82% contained no more than 10 CFU/ml. The ability of the L-C and broth systems to recover all organisms was not significantly affected by colony counts, but differences in the ability of the two systems to recover particular species or groups were most apparent in cultures with low colony counts. Virtually all of the cultures for members of the *Enterobacteriaceae* and yeasts in which the broth cultures were negative contained no more than 10 CFU/ml. Similarly, there was no difference in the recovery of streptococci when colony counts were >1 CFU/ml. Although the overall recovery of *Pseudomonas* spp. in broth and by L-C was not significantly different, the broth system was superior ($P < 0.05$) to L-C when colony counts were <1 CFU/ml.

Aerobic or facultative anaerobic organisms and the groups listed in Table 3 were recovered equally well on the three types of media incubated aerobically. All obligate anaerobes were isolated only from the anaerobic atmosphere.

With the exception of yeasts, organisms were usually detected the same day with each of the two culture systems, and isolated colonies were available 1 day earlier with the L-C system. The mean time to first detection of growth of all organisms was 1.5 days with the L-C system and 1.8 days with the broth system. The mean time to first availability of isolated colonies was 1.5 days with the L-C system and 2.6 days with the broth system. For yeasts, the mean times to first detection and first availability of isolated colonies with the L-C system were both 1.9 days, but they were 2.9 and 3.9 days, respectively, with

TABLE 3. Comparative recovery according to colonies per milliliter of blood

Organism or group	No. of isolates recognized when colony counts (CFU/ml) were:								
	<1			1–10		11–100		>100	
	L-C and broth	L-C only	Broth only	L-C and broth	L-C only	L-C and broth	L-C only	L-C and broth	L-C only
Enterobacteriaceae	31	38	24	46	10	18	0	7	2
Yeasts	13	24	6	22	5	13	1	7	0
Streptococcus spp.	12	8	19	15	0	4	0	2	0
Staphylococcus aureus	4	7	6	12	6	13	0	9	0
Pseudomonas spp.	7	4	12	7	1	3	0	1	0
Anaerobes	2	8	13	3	2	2	1	2	0
Other	1	5	9	5	2	1	0	1	0

TABLE 4. Number of organisms isolated from vented and unvented broth bottles

Organism or group	No. of organisms isolated from:		
	Vented bottle only	Unvented bottle only	Both bottles
Enterobacteriaceae	18[a] (10)[b]	21 (7)	87 (7)
Yeasts	41 (5)	0 (0)	20 (1)
Streptococcus spp.	13 (10)	5 (3)	34 (6)
Staphylococcus aureus	6 (2)	6 (3)	32 (1)
Pseudomonas spp.	5 (3)	3 (3)	22 (6)
Anaerobes	1 (1)	17 (11)	4 (1)
Other	5 (4)	2 (2)	10 (3)

[a] Total broth isolates.

[b] Values in parentheses are those isolated only from the broth system.

the broth system. The mean times to first detection and first availability of isolated colonies in the polymicrobic cultures were similar to those observed in cultures containing only one organism.

Contaminants were recovered from 8.2% of L-C cultures and 1.9% of broth cultures. During the 12 months of the study, the L-C contamination rate ranged from a monthly low of 1.6% to a high of 12.8%. Most of the contaminants on the L-C agar plates were one of two colonies of *S. epidermidis* with colony counts of <0.5 CFU/ml. However, low colony counts were also observed in cultures from patients with repeated *S. epidermidis* bacteremia. Of 23 cultures from 12 patients with 3 or more cultures positive for *S. epidermidis*, the colony counts were <0.5 CFU/ml in 8, 1 to 10 CFU/ml in 8, 11 to 100 CFU/ml in 5, and >100 CFU/ml in 2.

Organisms recovered from the vented broth bottle, the unvented broth bottle, or both are shown in Table 4. The vented bottle yielded significantly more yeasts ($P < 0.0001$) than did the unvented bottle, and significantly more anaerobes ($P < 0.0001$) were recovered from the unvented bottle.

DISCUSSION

In this study, significantly more total organisms, *E. coli*, and *Candida* spp. were recovered from the L-C system, and significantly more streptococci were recovered from the broth system. Neither system was superior for recovery of any other organism or organism group, but the total number of some species was too small for meaningful statistical analysis. For example, some slower-growing organisms such as *Bacteroides* spp., *Corynebacterium* sp. CDC-JK, and *Haemophilus* spp. were recovered more often from the broth system, but the differences were not statistically significant. The L-C system was also more effective in detecting patients with bacteremia and fungemia due to members of the *Enterobacteriaceae* and yeasts.

Most of the colony counts were low, and it was in these cultures with low colony counts that the superiority of either system was apparent. None of the overall superiority of the L-C system to recover members of the *Enterobacteriaceae* or yeasts or of the broth system to recover streptococci was apparent when colony counts were high. Moreover, statistically significant advantages of the broth system to recover *Pseudomonas* spp. were observed upon analysis of cultures containing low colony counts, but not of all positive cultures. It is not surprising that differing sensitivity of the two systems occurred in cultures with low colony counts. One would expect that any blood culture system should recover organisms when the initial inoculum is large and that greater sensitivity of a particular system would be observed primarily in cultures in which the initial inoculum is small.

Most bacteria were first detected at approximately the same time with both systems. However, with the L-C system, bacteria were isolated an average of 1 day earlier and yeasts were isolated an average of 2 days earlier. Earlier availability of isolated colonies on agar plates is a distinct advantage of the L-C system in cases of polymicrobic septicemia and enables the inoculum size to be standardized for organism identification and antimicrobial susceptibility testing.

The L-C system permits a choice of culture media, incubation atmospheres, and periods of incubation. During this study, chocolate, blood, and brain heart infusion agars were used. The superiority of the L-C system for recovery of yeasts could not be attributed to the use of brain heart infusion agar. However, this agar may help support the growth of fungi such as *Histoplasma capsulatum* that were not recovered during this study. A decision on media and culture conditions may be based on the organisms expected to be recovered from patients.

Contamination of L-C agar plates usually occurred as one or two colonies (<0.5 CFU/ml) of *S. epidermidis* on one of the four agar plates. However, the appearance of one or two colonies of *S. epidermidis* could not always be attributed to contamination. Many of the L-C cultures from patients who probably had true bacteremia, as indicated by three or more positive blood cultures, contained <0.5 CFU of *S. epidermidis* per ml. Thus, colony counts alone cannot be used to differentiate *S. epidermidis* contamination from bacteremia.

We attribute the wide variation in the monthly contamination rate, 1.6 to 12.8%, to the technique used in the culturing process. We believe that the contamination rate can be minimized by

using relatively dry agar plates, disinfecting the processing area and L-C tubes, and working in commotion-free areas as rapidly as possible. We did not process the tubes in any type of hood; however, this may be helpful.

Our results indicate that neither the L-C system nor a standard two-bottle broth system would yield optimal blood culture results in a hospital such as ours if used alone. The use of L-C alone would have resulted in the recognition of significantly fewer streptococcal and low-level *Pseudomonas* bacteremias, and the use of a broth system alone would have resulted in the recognition of significantly fewer yeast fungemias and *E. coli* bacteremias. Moreover, elimination of the aerobic broth bottle would have resulted in the recovery of fewer yeasts, and elimination of the anaerobic broth bottle would have resulted in the recovery of fewer obligate anaerobes. Whether the inherent ability of the L-C system to provide colony counts will be found to be useful in monitoring the response of a patient to treatment, whether these counts can be used to differentiate contaminants from pathogens, and how well the L-C system recovers some species that were seen only in small numbers in this study remain to be determined.

ACKNOWLEDGMENTS

The assistance of the technologists of the Microbiology Laboratory, Memorial Sloan-Kettering Cancer Center, is gratefully acknowledged. Helen Rigopoulos helped prepare the manuscript. We thank Aaron Duke for his invaluable aid in the statistical analysis of the data.

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