Rate of detection of bacteremia

Retrospective evaluation of 23,392 blood cultures

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Rapid detection of bacteremia is of primary importance to the clinical microbiologist. Recently there has been much interest in the development of blood culture methods which will increase the yield and decrease the time of detection of positives. Another major concern is the reported inadequacy of standard culture methods for the recovery of certain organisms such as *Pseudomonas*[1] and *Candida*.[1, 2] Research efforts currently are directed toward the development of more suitable media,[3] more effective blood culturing techniques,[4–7] and new detection systems.[8, 9]

This study was undertaken to determine actual detection times of bacteremia with generally accepted methods on a routine basis in The Cleveland Clinic Foundation Department of Microbiology. Parameters considered in the study were detection times, the number and type of organisms recovered, and the efficacy of blind subculturing.

Methods

The study made retrospectively included 23,392 blood cultures completed in 1975 by analysis of the blood culture worksheets on file in the Department of Microbiology. Blood culture procedures follow the recommendations of Bartlett et al[10] and are briefly outlined as follows:

1. A 2% solution of tincture of iodine was applied to the skin over the selected vein. The iodine was allowed to dry for 1 minute and removed with 70% isopropyl alcohol. The process was then repeated.

2. Ten milliliters of venous blood was drawn and 5 ml was transferred to each of two blood culture bottles. One bottle containing 50 ml of Trypticase Soy Broth (BBL) with 10% CO_2 and 0.025% sodium polyanetholsulfonate (SPS) was incubated aerobically at 35 C. The aerobic bottles were continuously vented by inserting a sterile airway (Travenol Laboratories). One bottle contained thioglycollate (BBL) with 10% CO_2, and 0.025% SPS was incubated anaerobically. After March 1975 the same procedure was followed using Difco Tryptic Soy Broth with 10% CO_2 and SPS 0.025% (vented), and Difco Thiol with 10% CO_2 and 0.025% SPS. The bottles were then taken to the laboratory and incubated at 35 C.

3. The blood culture bottles were examined daily for visible microbial growth. If growth was suspected, a Gram stain was made. Gram-positive organisms were plated onto two blood agar plates: one was incubated with 10% CO_2 tension, and the other was incubated anaerobically. Gram-negative organisms were plated as above and also plated onto a MacConkey agar plate which was incubated aerobically. Positive Gram stain results were recorded on the worksheets.

4. After 48 hours blood culture bottles not determined to be positive were "blind subcultured" to a blood agar plate which was incubated anaerobically and on a chocolate agar plate with 10% CO_2 tension.

5. Blood culture bottles that were not determined positive by "blind subculturing" were examined daily for 14 days and then discarded.

A total of 23,392 blood cultures were drawn involving approximately 47,000 culture bottles during 1975. The worksheets were examined for detection time of the individual organisms. Mean detection times and the median time of detection were determined for individual organisms. The number of organisms discovered at 3 days with the aid of "blind subculturing" was determined.

The organisms were then divided into two groups: probable pathogens and possible contaminants. Organisms usually thought of as ubiquitous saprophytes such as diphtheroids, *Bacillus* and *Staphylococcus epidermidis* were considered as possible contaminants when isolated from one of the two culture bottles. Also considered in this category were *Aspergillus*, *Penicillium*, and other molds. Organisms such as *Propionibacterium* may be contaminants but were not considered as such in this study. Organisms such as *S. epidermidis* may be considered pathogens in the rare case when recovered in successive blood cultures in one of the two culture bottles. The significance must be interpreted by the attending physician on an individual basis. Organisms not considered possible contaminants were considered probable pathogens.

Results

A total of 2835 organisms were recovered from 2635 total positive blood cultures and grouped on the basis of Gram stain reaction and morphology (*Table 1*). The 2635 total cultures which contained organisms were recovered from 23,392 blood cultures. From the 23,392 blood cultures drawn, approximately 47,000 culture media bottles were processed by the methods previously described. This involved approximately 4870 hours of technologist time and excluded clerical work and blood collection time. The 2635 positive cultures represented an 11.3% positive rate. More than one organism was isolated from 4.2% of the total positive cultures.

The mean time of detection for all recovered organisms was 4.6 days. The mean time of detection for the 750 organisms considered to be contaminants was 5.3 days. The mean time of detection for the 2095 organisms considered to be pathogens was 4.5 days. The median time of detection (time in which 50% of the organisms were recovered) for the organisms considered pathogens was 4 days and for the organisms considered to be contaminants 5 days.

Table 2 shows the efficacy of subculturing. Approximately 50% of the *Staphylococcus aureus, Pseudomonas aeruginosa,* and nearly 80% of the *Haemophilus* were recovered with the aid of subculture. *Table 3* lists the order of frequency of the recovered microorganisms. The contaminants *S. epidermidis* and diphtheroids were the most frequently recovered organisms. The mean detection time for the five most frequently encountered pathogens (*S. aureus, Escherichia coli, Pseudomonas, Bacteroides* and alpha-hemolytic streptococci) was 3.1 days, median time 2 days. (Data exclude *S. epidermidis* and diphtheroids.) Cumulative percent for the total recovered organisms is compared with the cumulative percent of the most frequently encountered pathogens in *Table 4*.

Discussion

The overall positive rate of 11.2% and the spectrum of organisms recovered were comparable to those reported in other series.[1, 3, 6, 8] The blood culture system used in the study provided rapid detection of the most frequently isolated pathogens as compared to times required for detection of the less commonly encountered pathogens and possible contaminants. Enterobacteriacae as a group have consistently short times of detection. Seventy-nine percent of the *E. coli*, 76% of the *Enterobacter*, 58% of the *Proteus*, and 57% of the *Klebsiella* were recovered in 2 days or less. The 48-hour subculture yielded 30% of all recovered organisms at a rate which was consistent with other reports.[1, 5] In the cases of *S. aureus, Candida* and *Pseudomonas,* subculturing proved even more valuable. Our data show that 39% of the *P. aeruginosa*, 50% of the *S. aureus,* and 76% of the *Candida albicans* were first recovered by subculturing.

Recovery of the 750 isolates classified as possible contaminants constituted 28% of the total positive cultures and 3.2% of the total cultures. The rate of recovery of contaminants was consistent with that found in other studies.[8] The time of detection of the 750 organisms considered as possible contaminants was skewed to the right of the mean and median times of detection. The recovery and

Table 1. Mean and median times of detection and frequency of isolates

Organism	No. recovered	Mean time of detection (days)	Median time of detection (days)
Gram-negative bacilli			
E. coli	282	2.1	2
P. aeruginosa	168	3.6	3
B. fragilis	156	3.0	2
Klebsiella	96	2.9	2
Enterobacter	56	2.3	2
Proteus	50	2.9	2
Acinetobacter	30	5.3	3
Serratia	21	4.6	3
Miscellaneous gram-negative bacilli*	18	4.9	3
Salmonella	15	3.1	3
Haemophilus	12	3.1	3
Citrobacter	7	4.3	3
Flavobacterium	3	8.0	8
Aeromonas	1	3.0	3
Alcaligenes	1	3.0	3
	916	2.9	2
Gram-negative cocci			
Neisseria	10	3.7	3
Gram-positive bacilli			
Diphtheroids	457	9.1	9
Bacillus	77	4.9	3
Clostridium	40	2.4	2
Propionibacterium	21	5.1	10
Miscellaneous gram-positive bacilli*	12	6.3	3
Listeria	11	3.1	3
	618	7.9	7
Gram-positive cocci			
S. epidermidis	615	5.1	3
S. aureus	253	3.0	3
Streptococci, alpha hemolytic	206	2.9	2
Enterococci	75	2.3	7
Streptococci, gamma hemolytic	21	3.6	3
Streptococci, beta hemolytic	17	3.5	2
Peptostreptococcus	14	5.9	5
Microaerophilic streptococci	12	6.3	5
Miscellaneous gram-negative cocci*	9	6.7	5
	1222	4.1	3
Molds			
Aspergillus	6	7.7	1
Penicillium	4	7.3	6
Mucor	2	8.5	8.5
Alternaria	1	11.0	11
Cephalosporium	1	9.0	9
Rhizopus	1	4.0	4
	15	7.7	–
Yeast			
Candida	51	5.5	3
Torulopsis	3	7.6	10
	54	5.6	3

* No further identification was made past Gram morphology.

Table 2. Comparison of detection times at 3 days

Organism	No. found on the 3rd day with the aid of blind subculture	No. found <3 days	No. found ≤3 days	Percent found ≤3 days	Percent found without aid of subculture <3 days
Gram-negative bacilli					
E. coli	33	223	256	90.1	79.1
P. aeruginosa	66	75	141	90.4	44.6
B. fragilis	59	63	122	78.8	40.4
Klebsiella	18	55	83	77.1	57.3
Enterobacter	9	43	52	92.9	76.8
Proteus	11	29	40	80.0	58.0
Acinetobacter	13	14	17	56.7	46.7
Serratia	8	10	18	85.7	47.6
Miscellaneous gram-negative bacilli*	7	3	10	55.6	16.7
Salmonella	5	6	11	73.3	40.0
Haemophilus	9	1	10	83.3	8.2
Citrobacter	3	2	5	71.4	28.6
Flavobacterium	1	0	1	33.3	0
Aeromonas	1	0	1	100.0	0
Alcaligenes	1	0	1	100.0	0
Gram-negative cocci					
Neisseria	7	0	7	70.0	0
Gram-positive bacilli					
Diphtheroids	37	15	52	11.4	3.3
Bacillus	26	14	40	51.9	18.2
Clostridium	9	27	36	90.0	67.5
Propionibacterium	1	0	1	4.8	0
Miscellaneous gram-positive bacilli	1	3	4	33.4	25.0
Listeria	6	4	10	90.9	36.4
Gram-positive cocci					
S. epidermidis	209	51	260	42.3	8.3
S. aureus	126	106	232	92.9	41.9
Streptococci, alpha hemolytic	67	98	165	47.6	64.8
Streptococci, gamma hemolytic	4	12	16	76.2	57.1
Streptococci, beta hemolytic	15	10	25	92.6	58.8
Peptostreptococcus	1	0	1	7.1	0
Microaerophilic streptococci	0	1	1	8.3	11.1
Miscellaneous gram-positive cocci	1	1	2	22.2	0
Molds					
Aspergillus	0	0	0	0	
Penicillium	0	0	0	0	
Mucor	0	0	0	0	
Alternaria	0	0	0	0	
Cephalosporium	0	0	0	0	
Rhizopus	0	0	0	0	
Yeasts					
Candida	22	7	29	58.0	
Torulopsis	0	0	0	0	

* No further identification was made past Gram morphology.

Table 3. Rank and relative percent incidences of isolates

Organism	No. recovered	Percent of the total positive blood cultures
S. epidermidis	615	21.69
Diphtheroids	457	16.12
E. coli	282	9.95
S. aureus	253	8.92
Streptococci, alpha hemolytic	206	7.26
P. aeruginosa	168	5.93
B. fragilis	156	5.50
Klebsiella	96	3.39
Bacillus	77	2.72
Enterococci	75	2.65
Enterobacter	56	1.97
Candida	51	1.79
Proteus	50	1.76
Clostridium	40	1.41
Acinetobacter	30	1.05
Propionibacterium	21	.74
Serratia	21	.74
Streptococci, gamma hemolytic	21	.74
Miscellaneous gram-negative bacilli*	18	.63
Streptococci, beta hemolytic	17	.59
Salmonella	15	.53
Peptostreptococcus	14	.49
Haemophilus	12	.42
Miscellaneous gram-positive bacilli*	12	.42
Microaerophilic streptococci	12	.42
Listeria	11	.39
Neisseria	10	.35
Miscellaneous gram-negative cocci*	9	.32
Citrobacter	7	.25
Aspergillus	6	.21
Penicillium	4	.14
Flavobacterium	3	.11
Torulopsis	3	.11
Mucor	2	.07
Aeromonas	1	.04
Alcaligenes	1	.04
Alternaria	1	.04
Cephalosporium	1	.04
Rhizopus	1	.04
	2835	100.00

* No further identification was made past Gram morphology.

Table 4. Comparison of cumulative percent for the 10 most frequent pathogens vs total isolates

Detection time, days	Total recovered organisms, %	Ten most frequently recovered pathogens, %
1	11.8	34.4
2	32.5	55.5
3	63.0	85.8
4	69.5	89.7
5	72.5	92.8
6	76.1	94.7
7	79.3	96.1
8	83.8	97.0
9	86.3	97.4
10	89.9	98.2
11	92.8	98.8
12	95.8	99.4
13	98.3	99.6
14	100.0	100.0

identification of contaminants contributed significantly to the overall blood culture work load.

As a result of this study and others, the blood culture procedure of The Cleveland Clinic Foundation Department of Microbiology was modified. Culture bottles are now held 7 days instead of 14, and all blood cultures not previously detected as positive are subcultured at 24 hours and then terminally subcultured at 7 days. The terminal subculture is held for 2 days. Holding bottles for the second week of incubation has resulted in slight gains in recovery, principally of contaminants as demonstrated in *Table 4*. The 24-hour subculture is expected to decrease the detection time in the cases of *S. aureus, Pseudomonas, Candida,* and highly fastidious organisms such as *Neisseria* and *Haemophilus*.

As a result of these modifications significant numbers of *P. aeruginosa*, *S. aureus*, and other organisms now are recovered more rapidly. The availability of biphasic culture media and Castenada bottles has also improved the recovery of *Candida* and other fastidious organisms.

Summary

The 23,392 blood cultures performed by The Cleveland Clinic Foundation Department of Microbiology for the 1975 calendar year were evaluated with respect to the number of organisms recovered, frequency, and species recovered. Mean and median times of detection were determined and a cumulative percent table was made. A total of 2635 positive cultures or 11.3% were recovered, 4.2% of which contained multiple organisms; 750 organisms were classified as possible contaminants representing 3.2% of the total cultures or 28% of the total positive cultures. The mean and median times of detection were 4.6 and 4 days respectively. *E. coli* and *S. aureus* were the most frequently recovered pathogens. Blind subculture was demonstrated to be an effective tool for detecting *Candida, Haemophilus, Neisseria,* and *Pseudomonas*. Modification of the routine procedures based on the results of the study are proposed.

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