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Evaluation of Two Commercially Available Media for Detection of Bacteremia

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Analysis of the results of 13,162 blood cultures during a 9-month interval has shown that *Pseudomonas aeruginosa* statistically was recovered more frequently from Trypticase soy broth (TSB) than from Thioglycollate-135C and that contaminants, including *Staphylococcus epidermidis* and aerobic and anaerobic *Corynebacterium* species, were isolated with statistically greater frequency from Thioglycollate-135C than from TSB. No other statistically significant differences were found.

In a previous publication from this laboratory two commercially available media, Thiol broth (Difco) and tryptic soy broth (Difco), were compared with respect to the number of isolates in positive cultures in each medium and with respect to time interval to positivity in each medium (2). The present report compares two other commercially available and commonly used broth media, Trypticase soy broth (TSB; Becton-Dickinson) and Thioglycollate-135C (Becton-Dickinson).

MATERIALS AND METHODS

From January 1971 through September 1971 all blood for routine cultures at the Mayo Clinic and affiliated hospitals was inoculated (10%, v/v) into one bottle each of TSB and Thioglycollate; each bottle contained 100 ml of medium under vacuum with added CO_2. The blood was collected aseptically with a sterile needle and syringe after skin preparation with 2% aqueous iodine and 70% isopropyl alcohol. Both bottles were incubated unvented at 35 C and were examined daily for 14 days. Routinely, at 24 hr subcultures were made, to chocolate-blood-agar plates (BioQuest), of bottles without visible evidence of growth; these were incubated in an atmosphere of 10% CO_2 at 35 C.

Identification procedures of isolates and statistical analysis were performed as described in the previous report (2).

RESULTS

During the months of study, 13,162 blood specimens were submitted to the microbiology laboratory. There were 1,116 positive cultures (8.5%), defined by positivity in one or both bottles in one set (Table 1), representing 566 patients (Table 2). By using the same arbitrary criteria for contamination described in the previous study, 269 (2.1%) of the cultures, representing 229 patients, were considered to be contaminated.

Two different bacterial species were isolated concurrently from each of 66 cultures; however, in 9 of these, one of the isolates was *Staphylococcus epidermidis* or *Corynebacterium* spp., and in five cultures the two isolates were *S. epidermidis* with *Bacillus* spp. or *Corynebacterium* spp. There were 13 cultures with three different bacterial species isolated from each; in two of these cultures, two of the isolates were *S. epidermidis* and *Corynebacterium* spp. Excluding patients with presumed contaminants in their blood cultures there were 35 patients (10%) with polymicrobial bacteremia.

Examination of all positive cultures (Table 1), by using Cochran's χ^2 test, showed that aerobic and anaerobic species of *Corynebacterium* and *S. epidermidis* were isolated from Thioglycollate significantly more frequently ($P < 0.01$ and $P < 0.05$, respectively) than from TSB, whereas species of *Pseudomonas aeruginosa* were significantly more frequently isolated ($P < 0.01$) from TSB than from Thioglycollate. In those instances in which cultures on both media became positive (Table 3), *Escherichia coli*, streptococci of the viridans group, and *S. epidermidis* were recovered more rapidly ($P < 0.01$ in each case) from Thioglycollate than from TSB; however, the time intervals to positivity in each medium differed by less than 1 day with *E. coli* and by only 1 day with the other two organisms.

To try to eliminate a bias in these analyses attributable to several positive cultures from the same patient with the same organism, sta-

TABLE 1. *Number of isolates in positive cultures, by media*[a]

Organism	No. positive				P (χ^2 analysis)
	TSB and Thio	TSB	Thio	Total positive	
Bacillus	1	10	11	22	NS[b]
Bifidobacterium	0	0	1	1	NS
Clostridium	3	0	4	7	NS
Corynebacterium	21	33	103	157	<0.01
Escherichia coli	123	59	43	225	NS
Salmonella	0	1	2	3	NS
Arizona	2	1	3	6	NS
Citrobacter	1	0	0	1	NS
Klebsiella	23	5	9	37	NS
Enterobacter	7	4	6	17	NS
Serratia	17	2	3	22	NS
Proteus	20	7	5	32	NS
Haemophilus	5	4	5	14	NS
Diplococcus pneumoniae	19	4	6	29	NS
Streptococcus, unspecified	1	0	1	2	NS
Streptococcus, viridans group	82	12	18	112	NS
Streptococcus, group A	11	3	1	15	NS
Streptococcus, group C	6	0	0	6	NS
Streptococcus, group D	25	10	8	43	NS
Streptococcus, other groups	3	0	0	3	NS
Eubacterium	0	2	2	4	NS
Herellea vaginicola	6	8	2	16	NS
Alcaligenes faecalis	4	3	3	10	NS
Mima polymorpha	0	0	1	1	NS
Neisseria	3	1	0	4	NS
Bacteroidaceae	54	20	18	92	NS
Micrococcus	1	1	1	3	NS
Staphylococcus aureus	60	19	32	111	NS
Staphylococcus epidermidis	28	22	40	90	<0.05
Peptostreptococcus	9	0	0	9	NS
Peptococcus	7	1	4	12	NS
Pseudomonas aeruginosa	42	27	4	73	<0.01
Candida	12	6	8	26	NS
Torulopsis glabrata	1	0	2	3	NS

[a] TSB = Trypticase soy broth; Thio = Thioglycollate.
[b] Not significant.

TABLE 2. *Number of patients positive, by organism*

Organism	No.	Organism	No.
Bacillus	14	*Streptococcus*, unspecified	2
Bifidobacterium	1	*Streptococcus*, viridans group	49
Clostridium	4	*Streptococcus*, group A	10
Corynebacterium	145	*Streptococcus*, group C	3
Escherichia coli	110	*Streptococcus*, group D	20
Salmonella	3	*Streptococcus*, other groups	1
Arizona hinshawii	2	*Eubacterium lentum*	4
Citrobacter freundii	1	*Herellea vaginicola*	11
Klebsiella pneumoniae	21	*Alcaligenes faecalis*	9
Enterobacter	9	*Mima polymorpha*	1
Serratia marcescens	10	*Neisseria meningitidis*	2
Proteus	20	*Bacteroidaceae*	43
Haemophilus	8	*Micrococcus*	3
Pseudomonas aeruginosa	31	*Staphylococcus aureus*	48
Diplococcus pneumoniae	12	*Staphylococcus epidermidis*	70
Candida	8	*Peptostreptococcus*	4
Torulopsis glabrata	2	*Peptococcus*	7

TABLE 3. *Time interval to positivity*

Organism	TSB[a]		Thio	
	No.	Days (mean)	No.	Days (mean)
Bacillus	11	7.2	12	7.0
Bifidobacterium	0	...	1	1.0
Clostridium	3	1.0	7	2.0
Corynebacterium	54	9.6	124	10.5
Escherichia coli	182	2.3	166	1.7
Salmonella	1	1.0	2	2.5
Arizona	3	5.6	5	1.4
Citrobacter	1	1.0	1	2.0
Klebsiella	28	2.3	32	2.1
Enterobacter	11	4.7	13	2.5
Serratia	19	1.8	20	2.0
Proteus	27	4.0	25	4.2
Haemophilus	9	5.1	10	4.6
Diplococcus pneumoniae	23	1.6	25	1.2
Streptococcus, unspecified	1	2.0	1	7.0
Streptococcus, viridans group	94	3.7	100	2.7
Streptococcus, ungrouped	1	3.0	0	...
Streptococcus, group A	14	1.1	12	1.3
Streptococcus, group C	6	1.8	6	1.5
Streptococcus, group D	34	2.4	32	2.6
Streptococcus, other groups	3	1.3	3	1.3
Eubacterium	2	2.5	2	8.0
Herellea vaginicola	14	1.9	8	3.0
Alcaligenes faecalis	7	3.4	7	3.6
Mima polymorpha	0	...	1	1.0
Neisseria	4	2.8	3	2.0
Bacteriodaceae	74	4.0	72	3.6
Micrococcus	2	11.0	2	8.5
Staphylococcus aureus	79	4.3	92	4.2
Staphylococcus epidermidis	50	6.9	68	6.3
Peptostreptococcus	9	4.8	9	2.6
Peptococcus	8	2.3	11	3.8
Pseudomonas aeruginosa	69	2.3	46	3.4
Candida	18	5.5	20	5.6
Torulopsis glabrata	1	2.0	3	8.3

[a] TSB, Trypticase soy broth; Thio, Thioglycollate.

tistical analyses were repeated using each patient's first positive culture only. By Cochran's χ^2 analysis, the same statistically significant differences with respect to the number of isolations of *Corynebacterium*, *S. epidermidis*, and *P. aeruginosa* from each medium were found; however, no statistically significant differences with respect to time interval to positivity were encountered.

DISCUSSION

The overall rate of positivity, excluding contaminants, of 6.4% in this study was lower than the 8% positivity rate, excluding contaminants, encountered previously (2). The reasons for this difference are not entirely clear. It is not known if Difco's tryptic soy broth differs to any significant degree from Becton-Dickinson's Trypticase soy broth. The smaller sample size in this study, plus the absence of the previously encountered statistically significant differences between the two media in the isolation of streptococci, may have contributed somewhat to this lower rate of positivity.

Cayeux et al. (1) recently described thiol-requiring mutants of streptococci isolated from heart valves or blood of three patients with endocarditis. The frequency of occurrence of this phenomenon and whether or not Difco's Thiol broth and Becton-Dickinson's Thioglycollate differ sufficiently in their content of thiol groups to account for the latter medium's apparently lesser efficacy in isolating streptococci are not known.

TSB continued to be the preferred medium for recovery of *P. aeruginosa*. The shorter time interval to detection of positivity of *P. aeruginosa* in TSB (mean, 2.3 days) in this study, in contrast to the previous one (mean, 4.1 days), is attributable, for the most part, to the shift from performing routine subcultures at 48 hr to performing routine subcultures at 24 hr. Finally, there were significantly more contaminants (*Corynebacterium* and *S. epidermidis*) grown from Thioglycollate than from TSB.

In this study, disregarding contaminants, anaerobic bacteria accounted for 13% of the positive cultures and 19% of the patients. These percentages are in close agreement with those reported previously (2). There were no statistically significant differences between TSB and Thioglycollate either in terms of the number of anaerobic bacteria recovered from each medium or in terms of the time interval for detection of the presence of anaerobic bacteria in each medium.

The time interval between blood culture collection and routine or "blind" subculture, the frequency of routine subcultures, and the subculture media and their conditions of incubation represent subjects on which there is little agreement between laboratories, as reported in a survey of 21 hospital laboratories conducted by Bartlett (Seminar on Bacteremia, American Society for Microbiology, Minneapolis, Minn., May 1971). In our experience, routine subcultures to chocolate-blood-agar have provided earlier recognition of blood cultures with *P. aeruginosa* than was possible by visual inspection of the cultures only. Shortening the time interval to subculture from 48 to 24 hr in this study did not alter the isolation rate of *P. aeruginosa* but did shorten the time interval to positivity in TSB. The question of whether routine subcultures to media incubated anaerobically would shorten the time intervals to positivity of *Bacteroidaceae* in TSB and Thioglycollate (4.0 and 3.6 days, re-

spectively) has not been studied.

In conclusion, under the conditions of this study, there were few differences noted between TSB and Thioglycollate-135C with the exception that *Pseudomonas aeruginosa* was more frequently isolated from TSB.

LITERATURE CITED

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